THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 80 | *December 1959* | NUMBER 6

PREVALENCE OF TUBERCULOSIS AMONG SELECTIVE SERVICE REGISTRANTS CURRENTLY PROCESSED FOR MILITARY SERVICE[1]

BERNARD D. KARPINOS

Received for publication June 23, 1959

Introduction

The remarkable progress made in the United States with respect to the control of tuberculosis (1, 2) is conspicuously reflected in the following analysis of the prevalence of tuberculosis among Selective Service registrants currently examined for military service. For a complete evaluation of such prevalence, the present analysis includes: cases diagnosed as tuberculosis among registrants examined for military service at the Armed Forces examining stations (AFES); cases of tuberculosis established by the local boards prior to the AFES examinations; and hospitalized cases of tuberculosis among registrants inducted into the Army, diagnosed as "existing prior to service" (EPTS). The AFES data will be discussed first.

Materials and Methods

Armed Forces Examining Stations Data

General: Each registrant forwarded by his local board to the Armed Forces examining stations (AFES) is evaluated as to his fitness for military service from a moral, mental, and medical (physical and psychiatric) standpoint (3). The medical evaluation consists of a complete medical examination, including a chest roentgenogram since 1953. (Prior to 1953 a chest roentgenogram was required only when the registrant was found otherwise medically qualified.) The findings of the medical examinations are reported on Standard Form 88 ("Report of Medical Examination").

Chest examination: The medical examination procedures and the standards of acceptability are stipulated in Army regulations (3, 4). In regard to chest examination, the current regulations require that a pertinent history of past chest diseases be taken in addition to the usual methods of physical diagnosis and a roentgenogram. The regulations emphasize particularly the importance of such an inquiry when certain symptoms of respiratory disorders are present, such as underweight, unexplained fever, recurring cough and expectoration, hemoptysis, pleurisy, or chronic laryngitis (4).

A small (70 mm.) roentgenogram is ordinarily used at the AFES. The film is read by a qualified roentgenologist, either civilian or military. If the interpretation of the small roentgenogram is difficult or doubtful, a 14- by 17-inch film is prescribed. Moreover, chest films showing doubtful shadows or nonacceptable lesions are jointly reviewed by the roentgenologist and an internist experienced in chest diseases. The original reporting is done on a roentgenographic report (Standard Form 519*a*) and is later transcribed by the Armed Forces examining stations to Standard Form 88 (item 46: Chest x-ray) (3).

Standards of acceptability: The chest findings are appraised in terms of the following standards of fitness for military service (4):

Acceptable: Profiles 1 and 2. Normal chest roentgenogram and no history of tuberculosis or pleurisy with effusion; discrete calcified lesions, single or multiple, hilar or parenchymal, with no history of active tuberculosis or of pleurisy with effusion. (Profiles 1 and 2 imply no restriction from a medical viewpoint as to the examinee's military assignment. *See* section "Coding Instructions" in regard to profiling.)

Acceptable: Profile 3. Roentgenographic finding of fibroid residuals not exceeding 4 sq. cm. in area, with or without calcified inclusions and with no history of tuberculosis or pleurisy with effusion, provided careful clinical study, including bacteriologic examinations and serial roentgenogram over a period of six months, shows no evidence of active disease; authenticated history of minimal tuberculosis more than five years previously with present chest films negative for parenchymal disease. (Profile 3 indicates certain assignment limitations.)

[1] From the Medical Statistics Division, Office of The Surgeon General, Department of the Army, Washington, D. C.

Nonacceptable: Profile 4. Active tuberculosis in any form or location; pulmonary tuberculosis, active within the past five years; spontaneous pneumothorax of tuberculous origin.

Sample studied: A copy of the medical examination report (Standard Form 88), including other pertinent records, of each disqualified registrant and a copy of these reports of each registrant inducted into the Army are forwarded to the Office of The Surgeon General of the Army. The data for this part of the analysis were taken from these reports. They relate to registrants who were examined during the fifteen-month period from January, 1957, through March, 1958.

During this period, 50 per cent of the received reports were coded. To ensure randomness of sample, the selection for coding was made by taking, for the disqualified registrants, all reports on which the Selective Service numbers assigned to the registrants by their local boards ended in an odd digit, and for the inductees, all reports on which the Armed Forces Service numbers ended in an odd digit. Of the coded reports, only those of registrants forwarded for a preinduction examination for the first time (not previously examined) were included in the study. (The reports of registrants forwarded for re-examination, usually registrants previously disqualified for medical causes, were excluded from the study for obvious reasons.)

The sample study included 91,000 reports of not previously examined registrants who were disqualified for military service (all reasons) and 86,000 forms of registrants who qualified for military service and were inducted into the Army.

The distribution of the reports by age, race, and military qualification of the examinees is presented in table 1. A per cent distribution of these examinees (disqualified and qualified) by single years of age and race is graphically shown in figure 1. The average age of the examinees was 21.5 years; this was also their modal age. The mean age was practically the same for both racial groups: white (21.6 years) and Negro (21.4 years). The term "white" denotes non-Negro.

Chest roentgenographic diagnostic categories: The following diagnostic categories were established for coding the chest roentgenographic findings as reported on the form:

1. Negative
2. Positive active tuberculosis
3. Arrested tuberculosis, acceptable for military service under the current medical standards for induction and enlistment
4. Arrested tuberculosis, not acceptable
5. Suspected active tuberculosis
6. Positive or suspected neoplasm
7. Positive or suspected circulatory pathology

TABLE 1

DISTRIBUTION OF THE MEDICAL EXAMINATION REPORTS OF SELECTIVE SERVICE REGISTRANTS INCLUDED IN THE SAMPLE, BY AGE, RACE, AND TYPE OF MILITARY QUALIFICATION OF THE EXAMINEES

(January, 1957, through March, 1958)

Age (as of Last Birthday)	Disqualified*		Inducted	
	White	Negro	White	Negro
Total	*66,663*	*24,894*	*75,936*	*10,029*
Less than 19	3,240	2,416	7,295	1,604
19–20	4,027	2,497	15,257	2,228
21–22	53,666	19,181	48,270	5,666
23–24	4,271	584	4,278	432
25 and older	1,459	216	836	99
Mean age (years)	22.1	21.6	21.4	21.3

* Relates to registrants forwarded for preinduction examination for the first time (not previously examined).

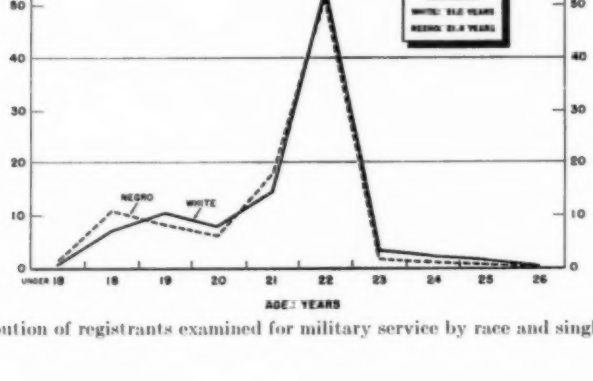

FIG. 1. Distribution of registrants examined for military service by race and single years of age.

8. Positive for chest pathology, other
9. Suspected chest pathology, other

Only one chest roentgenographic diagnostic code was used for each medical report. When more than one code was applicable to the chest roentgenographic findings, priority was given to the lowest numbered code, as listed above.

Coding instructions: The general instructions to the coders were that the chest roentgenographic entries (Standard Form 88, item 46) be appraised in terms of the following related entries on the form: "Lungs and Chest" (item 28), under which any associated abnormality is to be recorded; "Summary of Defects and Diagnoses" (item 74), under which all pertinent defects (whether disqualifying or not) are summarized; item 78 which lists the disqualifying defects whenever the examinee is found not acceptable; and the examinee's profile category (item 76).

The examinee's profile category presumably represents an evaluation of his functional capacity on the basis of the medical findings, in terms of the PULHES factors symbolizing briefly the following:

P—Physical capacity or stamina: General physical capacity or stamina, and organic defects or diseases which affect general physical capacity and which do not fall under the other specific factors of the profile system

U—Upper extremities: Functional use of hands, arms, shoulder, girdle, and spine (cervical, thoracic, and upper lumbar) in regard to strength, range of motion, and general efficiency

L—Lower extremities: Functional use of the feet, legs, pelvic girdle, lower back musculature, and lower spine (lower lumbar and sacral) in regard to strength, range of motion, and general efficiency

H—Hearing and ear defects: Auditory acuity and diseases and defects of the ear

E—Eyes: Visual acuity and diseases and defects of the eye

S—Psychiatric: Personality, emotional stability, and psychiatric diseases, including history of such

Each of the PULHES factors is profiled on a numerical regressive scale from 1 to 4. Generally, profile 1 represents functional efficiency above the average with no physical defects or with only minimal physical defects; profile 2 represents average functional efficiency, with mild, nonprogressive physical defects; profile 3 represents functional efficiency below the average with moderate physical defects (borderline); and profile 4 represents functional capacity below the existing minimum requirements for military service, that is, a disqualifying defect. (For more details, *see* (5, 6).) Any pathologic conditions of the lungs and chest would be accordingly profiled under the "P" factor; whenever the thoracic spine is affected, the condition would be usually profiled under the "U" factor.

The pertinent specific instructions are given here only with respect to the codes involving tuberculosis (codes 1 through 5). (A separate study is planned for the other pathologic conditions revealed by the chest roentgenographic findings.) These instructions to the coders are as follows:

Negative (*Code 1*). The code will be used when the chest x-ray entry clearly indicates normalcy. Examples of reporting: negative; NSA; normal; no pathology. This code will be used also in case of such entries, as small calcification; primary calcareous deposits, stabilized; primary Ghon tubercle; thickened pleura etc., provided no related pathology is reported in any of the other related items on the form.

Positive for active tuberculosis (*Code 2*). This code will be used when so specified in the chest x-ray entry, or when this entry reports pathology compatible with active tuberculosis and the "Summary of Defects and Diagnoses" contains concurrently a diagnosis of active tuberculosis or history of such. Examples of reporting: infiltration; infection; fibrous or soft lesion; spot; cloudy patch; necrosis, etc., when specified as tuberculous, or accompanied by a diagnosis of respiratory tuberculosis under "Summary of Defects and Diagnoses." Pleural effusion, when not specified as nontuberculous, will also be so coded. All such cases are profiled 4.

Arrested tuberculosis, acceptable (*Code 3*). This code will be used when so specified in the chest x-ray entry. These cases should have profile 3.

Arrested tuberculosis, not acceptable (*Code 4*). This code will be used when so reported in the chest x-ray entry, or when the x-ray findings are confirmed by a reported diagnosis or history of "old" or "arrested" tuberculosis. Examples of reporting: calcification; stable lesions; old rib resection; old pneumothorax; old empyema drainage scar, etc., specified as of tuberculous origin, or listed under "Summary of Defects and Diagnoses" as arrested, healed, or inactive tuberculosis, or history of tuberculosis.

Suspected active tuberculosis (*Code 5*). Examples of reporting: calcified tuberculous process, stability undetermined; increased density, rule out tuberculosis, etc., not supported by a definite diagnosis of tuberculosis (profile 4).

Observations

Distribution by chest roentgenographic findings: The distributions of the reports by chest roentgenographic findings and by race and type of the examinees' military qualification are presented in table 2. Fewer medical reports are shown in this table than in table 1 since no chest roentgeno-

TABLE 2

DISTRIBUTION OF THE MEDICAL EXAMINATION REPORTS BY CHEST ROENTGENOGRAPHIC FINDINGS AND BY RACE AND TYPE OF MILITARY QUALIFICATION OF THE EXAMINEES

(January, 1957, through March, 1958)

	Disqualified*		Inducted	
	White	Negro	White	Negro
Total	*64,377*	*23,974*	*75,365*	*9,956*
Tuberculosis:				
Negative	63,440	23,716	75,213	9,944
Arrested, acceptable	10	3	19	2
Positive, active	84	22	—	—
Suspected, active	78	23	—	—
Arrested, not acceptable	81	30	—	—
Other pathologic conditions:				
Total	684	180	133	10

* *See* footnote to table 1.

graphic entries were available for about 3.5 per cent of the disqualified registrants and for about 1 per cent of the qualified registrants. The chest roentgenograms were either not accomplished in these instances or their results were not recorded. This is true of both white and Negro registrants alike.

Prevalence of tuberculosis based on Armed Forces examining stations data: Based on the chest roentgenographic findings alone, the following prevalence rates of disqualifying respiratory tuberculosis were found among registrants examined at the AFES during the fifteen-month period: 13.1 per 10,000 total (white and Negro) examinees; 11.8 per 10,000 white examinees; and 19.9 per 10,000 Negro examinees (table 3). (*See* footnotes to table 3 on the computation of these rates.)

These prevalence rates include cases of tuberculosis diagnosed as positive active, suspected active, and arrested not acceptable.

In addition to the disqualifying cases of

TABLE 3

PREVALENCE OF TUBERCULOSIS AMONG SELECTIVE SERVICE REGISTRANTS EXAMINED AT THE ARMED FORCES EXAMINING STATIONS*

(January, 1957, through March, 1958)

Diagnosis	Number of Cases			Rates per 10,000 Examinees		
	Total	White	Negro	Total	White	Negro
Disqualifying tuberculosis	*494*	*380*	*114*	*20.3*	*18.5*	*30.2*
Chest roentgenographic findings	*318*	*243*	*75*	*13.1*	*11.8*	*19.9*
Positive, active tuberculosis	106	84	22	4.4	4.1	5.8
Suspected, active tuberculosis	101	78	23	4.1	3.8	6.1
Arrested tuberculosis, not acceptable	111	81	30	4.6	3.9	8.0
Other findings	*176*	*137*	*39*	*7.2*	*6.7*	*10.3*
Arrested respiratory tuberculosis, not acceptable	120	90	30	4.9	4.4	8.0
Nonrespiratory tuberculosis	56	47	9	2.3	2.3	2.3
Nondisqualifying tuberculosis	*52*	*46*	*6*	*2.1*	*2.2*	*1.6*
Arrested, acceptable	52	46	6	2.1	2.2	1.6
Population bases	242,919	205,218	37,701			

* Derived from table 2. The computations, both numbers and rates, were accomplished in the following manner. From the monthly reports ("Summary of Registrant Examinations for Induction," DA Form 316) submitted by the Armed Forces examining stations to the Office of The Surgeon General, Army, the following ratios of qualified to disqualified (not previously examined) registrants were established for this period: White: 2.188 (qualified): 1.000 (disqualified); Negro: 0.573 (qualified): 1.000 (disqualified). These ratios are obviously different from those of inducted to disqualified registrants in the sample. Consequently, to provide proper data for computing prevalence rates, the distributions of the inducted registrants by race (table 2) were weighted to agree with the above-given ratios of qualified to disqualified registrants. The necessary weighting factors were 1.869 and 1.379 for white and Negro inductees, respectively. The resulting weighted distributions of the inductees were combined with those of the disqualified registrants, as presented in table 2. The prevalence rates were calculated by relating the particular number of cases to the corresponding weighted population bases, as given at the bottom of this table.

respiratory tuberculosis established by the chest roentgenographic findings, a certain number of cases were diagnosed by the medical examiners of the AFES as disqualifying respiratory tuberculosis on the basis of other evidence, although the chest roentgenographic entries indicated normalcy. These were exclusively arrested not acceptable cases of respiratory tuberculosis. When the latter cases and those diagnosed as nonrespiratory tuberculosis are added to those diagnosed as disqualifying on the basis of chest roentgenographic findings, the following prevalence of disqualifying tuberculosis is obtained:

(*a*) Respiratory tuberculosis: Total (white and Negro)—18.0; white—16.2; Negro—27.9, per 10,000 examinees;

(*b*) Tuberculosis, all forms: Total (white and Negro)—20.3; white—18.5; Negro—30.2, per 10,000 examinees.

The data indicate a higher prevalence of disqualifying tuberculosis among Negro than among white examinees. This racial differential is seemingly due primarily to a relatively higher prevalence of suspected and arrested not acceptable cases of respiratory tuberculosis among Negroes (table 3). However, the actual difference between white and Negro registrants with respect to the prevalence of tuberculosis may not be as large as that reflected by the AFES data, as explained in the succeeding section.

Local Board Disqualifications

Prior to the examination by the Armed Forces examining stations, a certain prescreening is done at the local boards for the purpose of eliminating registrants with obviously disqualifying medical defects. In regard to tuberculosis, current available data on disqualifications at the local boards indicate that for each 17 registrants disqualified at the AFES for pulmonary tuberculosis, or for each 16 registrants disqualified for tuberculosis of all forms, 10 registrants are so disqualified at the local boards (7). The local board disqualification rates during this period were thus 10.7 for respiratory tuberculosis, or 12.4 for tuberculosis of all forms, per 10,000 registrants (white and Negro). These disqualifications consist of institutionalized cases of tuberculosis and cases of tuberculosis which the local board considered disqualifying on the basis of medical evidence furnished to them.

No ethnic breakdown is available for the local-board disqualifications. It is quite conceivable that relatively more white than Negro registrants are eliminated for tuberculosis by the local boards, as white registrants might possibly be more aware of the disease. Relatively more white than Negro registrants with tuberculosis are, therefore, likely to introduce evidence of the disease to the local boards. If this be true, rates by race based on AFES and local-board data, were it possible to obtain such data, might indicate smaller racial differences than those revealed by the rates based on AFES data alone.

The combined prevalence rates of disqualifying tuberculosis (white and Negro) were computed as 28.7 (18.0 at the AFES plus 10.7 at the local boards) for respiratory tuberculosis, or 32.7 (20.3 at the AFES plus 12.4 at the local boards) for tuberculosis of all forms, per 10,000 registrants examined for military service during the given period.

Cases of Tuberculosis after Induction

The pioneer studies of Yerushalmy and others (8–10) on roentgenographic findings of tuberculosis, followed by analogous studies by Long and others (11, 12) of the Army experience, and by Bircher and Castle (13) of the Navy experience, clearly demonstrated the existence of wide discrepancies in the interpretation of roentgenograms which undoubtedly result in failure to detect a certain number of respiratory cases of tuberculosis at the time of examination. Therefore, an attempt was made in this analysis to obtain data for estimating such failures among registrants examined for military service.

Toward this end, the punch card representing the individual medical records of Army patients were pulled for all cases of respiratory tuberculosis that were treated in Army hospitals during 1955 and 1956. These were cases in which respiratory tuberculosis was either the primary or the secondary diagnosis.

On the basis of the patients' Army Service numbers punched into the cards, it was possible to select the cases related to inductees. Next, the year of induction of each patient with respiratory tuberculosis was determined on the basis of the date of his admission to an Army hospital and his length of service at the time of admission. Only patients with respiratory tuberculosis identified as having been inducted in 1955 were considered. Their number amounted to 76.

The tour of military duty for inductees is two years. Obviously, for some of the 1955 inductees the tour of duty extended into 1957, so that some cases of respiratory tuberculosis from this group

TABLE 4

PREVALENCE OF DISQUALIFYING TUBERCULOSIS AMONG SELECTIVE SERVICE REGISTRANTS PROCESSED FOR MILITARY SERVICE
(January, 1957, through March, 1958)

	Rates
1. Local board disqualifications for tuberculosis (rate per 10,000 registrants):	
a. Respiratory	10.7
b. All forms	12.4
2. Disqualifications for tuberculosis at the Armed Forces examining station (rate per 10,000 registrants examined):	
a. Respiratory	18.0
b. All forms	20.3
3. Hospitalized cases of respiratory tuberculosis after induction (rate per 10,000 inductees)	7.3
4. Hospitalized EPTS cases of respiratory tuberculosis after induction (rate per 10,000 inductees)	4.1
5. Over-all prevalence rate of tuberculosis (rate per 10,000 registrants):*	
a. Respiratory	32.0
b. All forms	36.0

* As shown in the table (item 4), the prevalence rate of EPTS cases was 4.1 per 10,000 inductees. During the period of study, 8,046 were found medically qualified for service per 10,000 registrants examined. Hence, the prevalence rate of missed cases of tuberculosis per 10,000 registrants examined is 3.3 ($= 4.1 \times 0.8046$). The over-all rates become thus 32.0 for respiratory tuberculosis, or 36.0 for tuberculosis of all forms. Obviously, there could have also been a certain "over-reading" of the roentgenograms resulting in unjustified disqualifications. But this fact had to be disregarded here for lack of numerical data. It is also fully realized that, in computing the over-all rates, the 1955 experience of hospitalized cases was combined with 1957–1958 induction experience. This was not done by choice as those based on the 1955 experience were the most recent available data. It is believed, however, that the two- to three-year difference in time between these experiences could not appreciably affect these general results.

could have been admitted in 1957. (The 1957 data were not available for this study.) It was estimated from the 1955–1956 data that an additional 21 cases of respiratory tuberculosis could be expected in 1957 from the 1955 group of inductees. Thus, a total of 97 (76 ± 21) cases of respiratory tuberculosis was estimated for the 134,000 inductees in 1955.

Evidently, the current Army experience indicates that 7 of 10,000 inductees may be expected to be hospitalized for respiratory tuberculosis during their two-year tour of military duty (table 4, item 3).

Over-all Prevalence Rate

The 1955-1956 data further revealed that 57 per cent of these hospitalized cases of respiratory tuberculosis (4 cases per 10,000 examinees) were diagnosed as "existing prior to service" (EPTS). These are obviously cases of disqualifying respiratory tuberculosis missed at the time of the registrants' examination for military service.

By combining the EPTS cases of tuberculosis with the cases diagnosed as tuberculosis at the AFES and those disqualified for tuberculosis by the local boards, the over-all prevalence rates of disqualifying tuberculosis per 10,000 registrants currently examined for military service become 32.0 for respiratory tuberculosis, or 36.0 for tuberculosis of all forms. (*See* footnote to table 4 for the computation of these rates.)

These rates may be taken as the current prevalence rate of tuberculosis in the total male population within the 21- to 22-year age range.

Current versus World War II Prevalence

In order to evaluate the extent of the recent reduction in the prevalence of tuberculosis, the current prevalence data are compared with those of World War II (table 5). The comparison is made with respect to respiratory tuberculosis. The prevalence of disqualifying respiratory tuberculosis found at the induction stations among World War II registrants examined for military service was determined (14) as: 124.9 per 10,000 total (white and Negro) examinees; 118.2 per 10,000 white examinees; and 162.7 per 10,000 Negro examinees (table 5).[2] By including the local board disqualifications for tuberculosis, the total prevalence of disqualifying tuberculosis among World War II registrants was calculated as 135.8 per 10,000 examined registrants (table 5). The World War II prevalence rates were clearly very much higher than the current prevalence rates.

The present examinees, however, are younger than those of World War II: the age range of the present examinee is 18 through 26 years (mean age 21.5 years), as compared with the 18 through 37-year age range of World War II examinees (mean age 25.5 years). Since the prevalence of

[2] World War II data (14) comprise the period from January, 1943, through January, 1944.

TABLE 5

CURRENT AND WORLD WAR II PREVALENCE OF RESPIRATORY TUBERCULOSIS AMONG SELECTIVE SERVICE REGISTRANTS EXAMINED FOR MILITARY SERVICE

Item	Rates per 10,000 Examinees			
		Current		
	World War II*	Expected†	Actual‡	Per cent Decrease since World War II§
Excluding local board disqualifications:				
Total	*124.9*	*86.4*	*18.0*	*79.4*
White	118.2	80.7	16.2	79.9
Negro	162.7	118.7	27.9	76.5
Including local board disqualifications:				
Total	135.8	93.9	28.7	69.4

* Taken from B. D. Karpinos (14).

† "Expected" rates were standardized for age on the basis of World War II experience. The rates were derived by what is known as the "indirect" method of standardization. Briefly stated, this method is used in comparing rates of two or more groups whenever these groups differ with respect to some variable affecting the rates. In our case, the variable is age. The rates were computed by multiplying the age and race specific (expressed in two-year intervals) prevalence rates of tuberculosis in World War II (14) by the corresponding age distribution of the present examinees and summing the individual products to obtain a total "expected" rate for the particular racial group. The differences between the actual and the "expected" rates reflect actual differences, not affected by age. (For a detailed discussion in regard to this method of standardization, *see* (16).)

‡ Taken from tables 3 and 4.

§ Relates to "actual" over "expected."

tuberculosis generally increases with age (14, 15), a proper comparison requires rates of identical age ranges. This was accomplished by computing "expected" rates based on World War II experience. These rates (standardized for age) show what would have been the current prevalence rates of disqualifying tuberculosis had there been no change in the age specific prevalence rates since World War II (table 5). (*See* table 5, footnote†, on the derivation of "expected" rates.)

The "expected" rates of disqualifying respiratory tuberculosis were computed as 86.4, 80.7, and 118.7 per 10,000 total (white and Negro), white and Negro examinees, respectively, when confined to cases diagnosed at the examining stations. These data indicate that 79 per cent fewer cases of disqualifying respiratory tuberculosis are discovered now at the induction stations than in World War II for comparable age ranges. The present disqualification rate for respiratory tuberculosis at the induction stations is only 21 per cent of what it was for this age group in World War II. The decline in the disqualification rates at the examining stations since World War II was about the same for both races: 80 per cent for white examinees, and 77 per cent for Negro examinees.

By adding the local board disqualifications, the total "expected" prevalence rate of respiratory tuberculosis was computed as 93.9 per 10,000 registrants versus the actual current rate of 28.7, indicating a decline of 69 per cent since World War II in the disqualification rate for respiratory tuberculosis. (This percentage decrease is smaller than that indicated by the disqualification rates at the induction stations, as relatively more cases of respiratory tuberculosis are obviously screened out now by the local boards than in World War II.)

The prevalence rate of disqualifying respiratory tuberculosis among registrants currently examined for military service is thus somewhat less than one-third of what it was in World War II.[3]

There has been no basic change in the standards of acceptability of tuberculosis for military service between World War II and now. (*See* (15) on World War II standards.) Hence, the decrease in the disqualifying rate of tuberculosis for military service presumably reflects the decline in the prevalence of tuberculosis in the total population.

Factors Affecting Prevalence

Statistically, the prevalence of a disease, that is the proportion of a group of persons infected with that disease at a given time, depends on the following factors: (*a*) incidence, i.e., the rate at

[3] Since no adjustment was possible for hospitalized cases in World War II, as made for the current data, the comparison was made without this adjustment.

which the group is being infected with the particular disease—the lower the incidence, the smaller the prevalence and vice versa; (*b*) the efficacy of therapy—the higher the proportion of the infected persons being cured or becoming inactive, the lower the prevalence and vice versa; and (*c*) the length of time required for being cured or becoming inactive—the shorter the time, the lower the prevalence, and vice versa. These factors are, of course, interrelated as, in contagious diseases, more efficient and faster cure also decreases the chance for infecting others, which in turn decreases incidence. The striking decrease in the prevalence of respiratory tuberculosis among registrants obviously reflects the combined effects of all of these factors, namely, lower incidence of the disease, more effective therapy, and shorter time required for such therapy.[4]

A special follow-up study conducted by the Army with respect to Army personnel who were placed on the temporary disability retired list (TDRL) during 1950–1955 because of tuberculosis showed that 81 per cent of these persons were found fit for duty within one to two years after they had been placed on the TDRL, and only 2.3 per cent of them showed reactivation of the disease (17). Similar results are indicated by other sources (18). But, besides the current successful therapeutic measures (chemotherapy and improved surgical techniques) in the treatment of tuberculosis (19), there are other factors that are responsible for the gains in the control of this disease. These factors are, as Dublin (1) indicates, ever-increasing facilities for case finding; extensive development of tuberculosis clinics and sanatoriums; improved living conditions; and, generally, intensive "antituberculosis" campaigns.

Summary

The analysis presented here on the prevalence of tuberculosis among Selective Service registrants currently processed for military service is based on: cases of tuberculosis discovered at the Armed Forces examining stations (AFES) at the time when the registrants are examined for military service; cases of tuberculosis established by the local boards prior to the AFES examinations; and hospitalized cases of tuberculosis among inductees diagnosed as "existing prior to service" (EPTS).

The AFES data relate to registrants examined during the fifteen-month period from January, 1957 through March, 1958. It is a sample study comprising 177,000 examinees. The examinees during this period were within the age range of 18 through 26. Their mean and modal ages were 21.5 years.

Based on roentgenographic and other findings, the following prevalence rates of disqualifying tuberculosis were found among these examinees: (*a*) Respiratory tuberculosis: total (white and Negro)—18.0; white—16.2; Negro—27.9, per 10,000 examinees; (*b*) tuberculosis, all forms, total (white and Negro)—20.3; white—18.5; Negro—30.2, per 10,000 examinees.

The higher prevalence rates among Negro examinees appear to be due mainly to higher prevalence of suspected and arrested not acceptable cases of tuberculosis.

In addition to the cases of tuberculosis discovered at the Armed Forces examining stations, 10.7 per 10,000 registrants were screened out by the local boards for respiratory tuberculosis, or 12.4 for tuberculosis of all forms, prior to examination for military service. These rates include institutionalized cases and cases of tuberculosis established on the basis of medical evidence supplied to the local boards.

The prevalence rates of disqualifying respiratory tuberculosis were thus computed as 28.7, or 32.7 for tuberculosis of all forms, per 10,000 (white and Negro) registrants, based on the AFES and the local board disqualifications.

As demonstrated by several studies, a certain number of cases of tuberculosis are missed on the medical examinations. Consequently, a special study was undertaken to determine the number of registrants hospitalized for respiratory tuberculosis after induction. It was found that 4 of 10,000 inductees can be expected to be hospitalized during their two-year tour of military duty for respiratory tuberculosis, diagnosed as existing prior to service (EPTS). These pre-

[4] Obviously, a low prevalence could also result from high fatality; however, this is not the case here, since mortality due to tuberculosis has been rapidly decreasing. As calculated from Vital Statistics Reports, the mortality from respiratory tuberculosis declined from 39.6 in World War II (1941–1945) to a current (1956–1957) rate of 1.2 per 100,000 males in the 20- to 24-age group. The corresponding declines by race were from 24.1 to 0.6 for white and from 164.7 to 6.7 for nonwhite males. (For further details, *see* (1).)

sumably represent cases of respiratory tuberculosis missed at the time of the registrants' examinations for military service.

When adjusted for these latter cases, the overall prevalence rates become 32.0 for respiratory tuberculosis, or 36.0 for tuberculosis of all forms, per 10,000 registrants currently examined for military service. These rates may be taken as representative of the current prevalence of tuberculosis among the 21- to 22-year age group in the general male population.

The data indicate that the present prevalence of respiratory tuberculosis among Selective Service registrants is somewhat less than one-third of what it was in World War II within a comparable age range.

Sumario

Frecuencia de la Tuberculosis entre los Registrados en el Servicio Selectivo y Actualmente en Tramitación para el Servicio Militar

El análisis aquí presentado sobre la frecuencia de la tuberculosis entre los sujetos registrados en el Servicio Selectivo y actualmente en vías de tramitación para el servicio militar se basa en: los casos de tuberculosis descubiertos en el Puesto de Examen de las Fuerzas Armadas en la fecha en que se examina a los reclutas para el servicio militar; los casos de tuberculosis establecidos por las juntas locales de reclutamiento antes de los exámenes en el Puesto de Examen; y los casos hospitalizados de tuberculosis entre los reclutados, diagnosticados como "existentes antes del servicio."

Los datos del Puesto de Examen comprenden a los registrados examinados durante el período de quince meses desde enero de 1957 hasta marzo de 1958, inclusive. Es un estudio de una muestra que comprende 177,000 exámenes. Los examinados durante ese período eran de una edad que variaba de 18 a 26 años inclusive, teniendo una edad media y modal de 21.5 años.

A base de los hallazgos radiográficos y otros, se descubrieron las siguientes tasas de tuberculosis inhabilitante entre los examinados: (*a*) tuberculosis del aparato respiratorio: total (razas blanca y negra)—18.0; raza blanca—16.2; raza negra—27.9 por 10,000 examinados; (*b*) tuberculosis, todas formas, total (razas blanca y negra)—20.3; blanca—18.5; negra—30.2 por 10,000 examinados.

Las tasas más altas entre los examinados de raza negra parecen deberse principalmente a una frecuencia mayor de casos sospechosos o "inaceptables" estacionados de tuberculosis.

Además de los casos de tuberculosis descubiertos en los Puestos de Examen de las Fuerzas Armadas, 10.7 por 10,000 registrados fueron descartados por tuberculosis del aparato respiratorio por las juntas locales, o 12.4 por tuberculosis de todas formas, antes del examen para el servicio militar. Estas tasas comprenden los casos institucionalizados y los de tuberculosis establecidos a base de los datos médicos facilitados a las juntas locales.

Las tasas de frecuencia de tuberculosis inhabilitante del aparato respiratorio fueron computados de ese modo a 28.7, y a 32.7 para tuberculosis de todas formas, por 10,000 registrados (razas blanca y negra), a base de las inhabilitaciones de los Puestos de Examen y de las juntas locales.

Según demuestran varios estudios, en los exámenes médicos se pasa por alto cierto número de casos de tuberculosis. Por ello, se emprendió un estudio especial para determinar el número de reclutas hospitalizados por tuberculosis del aparato respiratorio después del alistamiento. Se descubrió que cabe esperar que se hospitalicen 4 de cada 10,000 reclutas durante su turno de 2 años de servicio militar por tuberculosis del aparato respiratorio, diagnosticada como existiendo antes del servicio. Estos representan presuntamente casos de tuberculosis del aparato respiratorio pasados por alto en los exámenes de los reclutas para el servicio militar.

Una vez ajustadas para estos últimos datos, las tasas globales de frecuencia suben a 32.0 por tuberculosis del aparato respiratorio, o 36.0 por tuberculosis de todas formas, por 10,000 reclutas examinados corrientemente para el servicio militar. Pueden tomarse estas tasas como típicas de la frecuencia actual de la tuberculosis en el grupo de veintiún a veintidós años de edad de la población masculina general.

Indican los datos que la actual frecuencia de la tuberculosis del aparato respiratorio entre los registrados en el servicio Selectivo es algo inferior a un tercio de lo que fuera en la Guerra Mundial II en un grupo de edad comparable.

Resume

Fréquence de la tuberculose parmi les conscrits examinés pour le Service Militaire

Cette étude sur la fréquence de la tuberculose parmi les conscrits est basée sur les cas de tuberculose décelés à l'"Armed Forces Examining Station (AFES)" au moment où ceux-ci étaient examinés en vue du service militaire; les cas de tuberculose décelés par les commissions locales, avant les examens de l'AFES; et les cas des conscrits hospitalisés, attients de tuberculose, diagnostiquée comme "existant avant le service" (EPTS).

Les renseignements recueilles par l'AFES portent sur des conscrits examinés pendant 15 mois, de

janvier 1957 à mai 1958. C'est une étude par sondage, portant sur 177.000 sujets. Au cours de cette période, l'âge des sujets examinés variait de 18 à 26 ans, l'âge moyen étant de 21 ans ½.

En se basant sur les radiographies et autres examens, les taux de fréquence de tuberculose entraînant l'élimination du candidat au service militaire furent les suivants: (a) tuberculose pulmonaire, total (blancs et noirs): 18,0, blancs: 16,2, noirs: 27,9, par 10.000 sujets examinés; (b) tuberculose, toutes formes, total (blancs et noirs) 20,3, blancs: 18,5, noirs 30,2 par 10.000 sujets examinés.

Les taux de fréquence plus élevés chez les sujets noirs semblent être dûs surtout à la fréquence plus élevée des cas de tuberculose suspectée ou inactive, "non-acceptables" pour le service.

En plus des cas de tuberculose découverts à l'AFES, 10.7 pour 10.000 conscrits furent éliminés pour tuberculose pulmonaire par les commissions locales, ou 12.4 pour tuberculose toutes formes, avant de subir les examens du service militaire. Ces taux comprennent les cas hospitalisés et les cas de tuberculose basés sur la preuve médicale fournie par les commissions locales.

Les taux de fréquence de la tuberculose pulmonaire entraînant l'élimination furent donc de 28,7 ou 32,7 pour la tuberculose toutes formes, par 10.000 conscrits (blancs et noirs), ces taux étant basés sur les éliminations faites par les AFES et les commissions locales.

Ainsi que de nombreux travaux l'ont démontré, un certain nombre de cas de tuberculose ne sont pas reconnus à l'examen médical. En conséquence une étude spéciale fut entreprise pour déterminer le nombre de conscrits hospitalisés pour tuberculose pulmonaire après leur recrutement. Il a été constaté que l'on pouvait s'attendre à ce que quatre recrus sur 10.000 soient hospitalisés pendant leurs deux années de service militaire pour tuberculose pulmonaire, existant avant le service (EPTS). Ceux-ci représentent vraisemblablement les cas de tuberculose pulmonaire qui n'ont pas été diagnostiqués lors de l'examen médical des conscrits.

Si l'on tient compte de ces derniers cas dans les résultats, les taux généraux de fréquence deviennent 32,0 pour la tuberculose pulmonaire ou 36,0 pour la tuberculose toutes formes par 10.000 conscrits examinés. Ces taux peuvent être considérés comme représentant la fréquence de la tuberculose dans toute la population masculine, de vingt-et-un à ving-deux ans.

Ces résultats montrent que la fréquence actuelle de la tuberculose pulmonaire parmi les conscrits est d'environ un tiers moins élevée qu'elle ne l'a été pendant la Deuxième Guerre Mondiale pour un groupe d'âge comparable.

Acknowledgments

The writer is indebted to Dr. Esmond R. Long; Col. H. E. Griffin; and Col. W. H. Moursund, Major P. E. Cevey, Captain D. E. Sullivan, Eugene L. Hamilton, and Arthur J. McDowell, Office of The Surgeon General, Department of the Army, for their critical reviews of the manuscript; to Anton Druzina and Aurelia Brumbaugh, Chief and Assistant Chief of the Induction Branch, Medical Statistics Division, respectively, for their faithful coding and collection of the induction data; to Karl Benson and Pauline Burroughs for the preparation of the material on the hospitalized cases of tuberculosis; to Helen Reeves for reading the manuscript; and to Eurselene J. Martin for her careful typing of the manuscript.

REFERENCES

(1) Dublin, L. I.: The course of tuberculosis mortality and morbidity in the United States, Am. J. Pub. Health, 1958, *48*, 1439.

(2) Glaser, S., Trauger, D. A., and Wyman, A. H.: Estimate of tuberculosis prevalence in the United States, 1956, Pub. Health Rep., 1957, *72*, 963.

(3) Department of the Army, Personnel Procurement: Armed Forces induction and examining stations, Army regulations 601–270.

(4) Department of the Army, Medical Service: Physical standards and physical profiling for enlistment and induction, Army regulations 40–503.

(5) Karpinos, B. D.: Evaluation of physical fitness of present-day inductees, U. S. Armed Forces M. J., 1953, *4*, 415.

(6) Karpinos, B. D., and Souther, G.: Limiting defects of Army inductees in physical categories B and C, U. S. Armed Forces M. J., 1956, *7*, 1791.

(7) Karpinos, B. D.: Fitness of American youth for military service (in manuscript form).

(8) Yerushalmy, J., Harkness, J. T., Cope, J. H., and Kennedy, B. R.: Role of dual reading in mass radiography, Am. Rev. Tuberc., 1950, *61*, 443.

(9) Garland, L. H.: On the scientific evaluation of diagnostic procedures, Radiology, 1949, *52*, 309.

(10) Yerushalmy, J., Garland, L. H., Harkness, J. T., Hinshaw, H. C., Miller, E. R., Shipman, S. J., and Zwerling, N. B.: An evaluation of the role of serial chest roentgenograms in estimating the progress of diseases in patients with pulmonary tuberculosis, Am. Rev. Tuberc., 1951, *64*, 225.

(11) Long, E. R., and Jablon, S.: Tuberculosis in the Army of the United States in World War II, Veterans Administration Medical Monograph, U. S. Government Printing Office, Washington 25, D. C., 1955.

(12) Long, E. R., Stein, S. C., and Henderson, H. J.: Experience with dual making of

chart photoroentgenograms, U. S. Armed Forces M. J., 1956, *7*, 493.

(13) Bircher, J. L., and Castle, C. A.: Evaluation of photofluorographic chest surveys in U. S. Navy, U. S. Armed Forces M. J., 1955, *6*, 951.

(14) Karpinos, B. D.: Prevalence of tuberculosis among registrants processed for military service, World War II (in manuscript form).

(15) Karpinos, B. D.: Prevalence of pulmonary tuberculosis among selectees, Human Biol., 1944, *16*, 181.

(16) Stouffer, S. A.: Standardization of rates where specific rates are unknown, Handbook of Statistical Methods for Demographers (edited by A. J. Jaffe), U. S. Department of Commerce, Bureau of the Census, 1951, p. 56.

(17) Wier, J. A., and Tempel, C. W.: Resumption of military duty after treatment of tuberculosis, U. S. Armed Forces M. J., 1957, *8*, 1814.

(18) Surgeon General, Army: Medical Statistics of the United States Army (Separations for Disability), Annual Reports, 1953, 1954.

(19) Mayock, R. L., Burke, R. M., Pinney, C. T., Gregory, L. J., and Wier, J. A.: New trends in the treatment of tuberculosis, U. S. Armed Forces M. J., 1955, *6*, 35.

MEASUREMENTS OF THE PULMONARY DIFFUSING CAPACITY FOR OXYGEN DURING EXERCISE[1,2,3]

ARTHUR A. SIEBENS,[4] N. ROBERT FRANK, DONALD C. KENT, MELVIN M. NEWMAN, ROBERT A. RAUF, AND BETTY L. VESTAL

(Received for publication January 12, 1959)

Introduction

The pulmonary diffusing capacity is interpreted to reflect the area and permeability of the alveolar-capillary "membrane" (1, 2). This interpretation, particularly as it applies to changes in area, is based principally on the logic of methods used for measuring diffusing capacity rather than on data either bearing out this interpretation or at variance with it. The present study is an attempt to obtain such data by measuring diffusing capacity under circumstances thought to affect primarily the area or primarily the permeability of the alveolar-capillary "membrane."

Selected patients who had resections of lung tissue were among those thought to have abnormalities primarily of area, and others with interstitial fibrosis and sarcoid disease were assumed to have defects primarily of permeability. The question arises among patients with resections whether their anatomic loss is associated with a commensurate physiologic loss, i.e., whether additional capillaries open around remaining alveoli in sufficient profusion to preserve the original dimensions of the gas-exchanging surface. The increase in pulmonary vascular resistance demonstrated following large resections (3, 4) indicates attenuation at some point in the lesser circulation and therefore suggests that such preservation is incomplete. This observation does not, however, localize the site of attenuation to the capillary network. Diffusing capacity is in concept a measurement capable of this localization. Added interest was therefore attached to its performance in patients with resections.

Methods

The pulmonary diffusing capacity for oxygen is the quotient obtained by dividing the volume (ml.) of oxygen diffusing from alveolar gas to corpuscular hemoglobin in one minute by a mean value for the pressure under which diffusion occurs (alveolar-capillary oxygen tension difference in mm. of mercury). The method of Lillienthal, Riley, Proemmel, and Franke (5) was used throughout. Alveolar tensions were calculated from the "alveolar equation" and capillary tensions from measurements of arterial tension performed at two levels of oxygenation. The charts of Riley and associates (6, 7) were used in quantifying components of the alveolar-arterial oxygen tension difference and in deriving the mean alveolar-capillary gradient from the gradient measured at the end of the capillary. The smallest end-gradients were 8 (one patient) and 9 (one patient) mm. of mercury; they were 12 mm. of mercury or higher in all but six studies. Mean gradients were 22 mm. or more with two exceptions (21 and 17 mm.). Mixed venous blood was assumed to be 40 per cent less saturated than end-capillary blood. The reader is referred to Comroe and associates (2) for further description of the method and to Riley and coworkers (1) for an analysis of its limitations.

The tensions of carbon dioxide and oxygen in arterial blood were measured by the bubble equilibration technique of Riley, Proemmel, and Franke (8, 9). Proficiency was obtained by working with arterial blood equilibrated with gases of known composition in a tonometer rotated in a temperature-controlled water bath. Empirical correction factors at selected tensions agreed with those published and the latter were therefore adopted. The same individual performed all tension measurements throughout the period of this study. Among 55 measured values, each the mean of two syringes, the difference between group means and values within the group was 1.41 ± 0.94 for carbon dioxide and 1.37 ± 1.22 for oxygen. In this exercise and in the patient studies, values were discarded in the rare instances in which tension differences between duplicate syringes exceeded 6 mm. of mercury.

Exercise was performed on a motor-driven

[1] From the Cardiopulmonary Laboratory, U. S. Naval Hospital, St. Albans, New York; and the Department of Physiology, State University of New York, Brooklyn, New York.

[2] Preliminary publications are indicated by references (37) and (38).

[3] This project was aided by a contract between the Office of Naval Research, Department of the Navy, and the State University of New York. Reproduction of this paper in whole or in part is permitted for any purpose of the United States Government.

[4] Present address: Respiratory and Rehabilitation Center, departments of Pediatrics and Physiology, University Hospitals, Madison, Wisconsin.

treadmill running at a speed (3.5 to 4.5 m.p.h.) and inclination (3° to 3.5°) which were thought to produce nearly the maximal exercise which the patient could sustain for twelve or thirteen minutes. A two- or three-minute sample of expired air was collected in a 600-liter spirometer which had been flushed with expired air six or seven times during the first ten minutes of exercise. After being mixed for three minutes, this collected gas was sampled and was analyzed with the Scholander apparatus (10). Blood samples were slowly drawn into heparinized and greased syringes through a Cournand needle placed in the brachial artery. Blood gas contents were measured by the method of Van Slyke and Neill (11). The oxygen-poor mixtures were inhaled through a low resistance valve from a previously flushed Douglas bag filled from a gas cylinder. The subject was allowed to rest for about one hour between bouts of exercise at the two levels of oxygenation. All studies of diffusing capacity were performed in the post-absorptive state.

The vital capacity and its subdivisions were measured in the supine position with a spirometer. A modified Darling open-circuit method was used for measuring the functional residual capacity (12); tabulated values are the mean of two determinations which agreed within 10 per cent. The maximal breathing capacity was performed in the sitting position by breathing through a low resistance valve into a Douglas bag for fifteen seconds. Lung volumes and ventilation were expressed at ambient pressure and saturated with water vapor; predicted values were calculated from the formulas of Baldwin and co-workers (13). Bronchospirometry was performed in the supine position through a Carlens tube inserted under 1 per cent tetracaine (Pontocaine®) topical anesthesia and positioned under fluoroscopic visualization.[5]

Most of the patients were young men on active duty in the armed forces stationed or hospitalized at the U. S. Naval Hospital, St. Albans; a few were dependents and retired personnel. The following groups of patients were included: 12 normal young men (table 1); 7 young men with bronchiectasis studied preoperatively (table 2); 16 young men studied after pulmonary resection. Four of these were studied following excision of an isolated pulmonary nodule (coin lesion) (table 3) and 12 following larger resections, all but two of which were performed for bronchiectasis (table 4); 10 patients with preoperative lesions thought either to affect primarily the area of the alveolar-capillary bed or to be of special interest (table 5); 7 young men with generalized disease of the alveolar-capillary membrane, i.e., sarcoid disease, interstitial fibrosis, and pulmonary fibrosis of unspecified etiology. The data were used in assessing changes in diffusing capacity due to bronchiectasis, to reducing alveolar-capillary surface area by surgical excision, to reducing alveolar-capillary surface area independently of surgical excision, and to reducing (it is presumed) primarily permeability and thickness of the alveolar-capillary "membrane."

It was recognized that it is impossible to distinguish with certainty those patients having changes exclusively in the area of the "membrane" and those with changes only in its permeability, this distinction probably being least reliable in the group of patients with "membrane disease." It appeared reasonable to assume, however, that the patients selected for these studies had predominantly either a reduction in area or predominantly a decrease in permeability of their alveolar-capillary bed. The subjects included in group comparisons were men of comparable age (14). They were studied at metabolic rates exceeding 1,298 ml. of oxygen per minute, values at which diffusing capacity approaches its higher limit (1, 15). In addition to the group data, individual studies were included which pertained to the effects of metabolic activity, age, and sex on diffusing capacity.

Abbreviations appearing in the tables are conventional (16).

Results

"Normal" Values (Table 1)

In a group of 12 men aged nineteen to thirty-five years, the diffusing capacity ranged from 68 to 86, the mean being 74.9 ml. per min. per mm., and the standard deviation 6.1. The diffusing capacity per square meter of body surface area ranged from 35 to 46, the mean and standard deviation being 39.7 ± 3.4 (figure 1). Three of these 12 subjects had no known pulmonary disease, whereas 6 had "coin" lesions and 3 had minimal infiltrates. Inclusion of these latter 9 in the group of "normal" subjects was based on the absence of respiratory complaints, the finding of a normal vital capacity, and, particularly, on the assumption that the circumscribed appearance and small dimensions of the lesions, as judged from roentgenograms, precluded their affecting the diffusing capacity detectably. The age range in this group was nineteen to thirty-five years; the lowest metabolic rate was 1,299 ml. per min., and the highest was 1,966 ml. per min.

The diffusing capacity of a normal active man aged sixty years was 40 or 21 per square meter of body surface, a value which is almost one-half the mean for the younger age group. It was obtained at a level of exercise (Vo_2 = 1,936) which compared with the highest achieved in the younger age groups.

One middle-aged woman with an isolated pulmonary nodule, although exercised apparently

[5] The data pertaining to lung volumes, ventilation and bronchospirometry are available from the writers.

TABLE 1

Clinical, Gas Exchange, and Diffusing Capacity Data of Normal* Subjects

Clinical Data				Basic Data										Derived Values			
Patient Number	Diagnosis	Age	B.S.A.	BP-47	$F_{I_{O_2}}$	$\dot{V}$	f	$F_{E_{CO_2}}$	R	$P_{a_{CO_2}}$	$P_{A_{O_2}}$	$P_{a_{O_2}}$	$\dot{V}_{O_2}$	$P_{A_{O_2}} - \bar{P}_{CO_2}$	Venous Admixture ($\dot{Q}_{VE}/\dot{Q}_T \times 100$)	Diffusing Capacity	
																Total	M²
		years	*M²*		*mm. Hg*	*l*				*mm. Hg*	*mm. Hg*	*mm. Hg*	*ml.*	*mm. Hg*			
12 Men, Aged 19–35																	
1168	Minimal infiltrate, left lower lobe	19	1.83	714	0.2093	44.7	29.6	5.073	0.84	44	99	82	2,230	24	4		
				714	0.1276	48.2	32.5	4.027	0.95	37	52	35	1,685	24	4	70	38
0549	Normal	20	1.63	714	0.2093	58.7	34.5	3.106	0.87	30	115	94	1,732	25	5		
				714	0.1344	53.5	30.0	3.605	0.90	34	59	46	1,760	25	5	70	43
0875	Coin lesion	20	1.83	715	0.2093	58.9	38.5	3.706	0.94	39	109	94	1,901	21	4		
				715	0.1254	66.1	35.5	3.176	1.17	39	56	35	1,475	21	4	71	39
0815	Coin lesion	21	1.90	716	0.2093	41.8	30.3	4.670	0.91	45	101	86	1,766	22	4		
				716	0.1257	62.1	35.0	4.174	1.17	38	56	44	1,821	22	4	83	44
0980	Coin lesion	22	1.86	715	0.2093	42.5	31.5	4.279	0.85	39	105	90	1,762	22	3		
				715	0.1175	62.7	38.0	3.549	0.97	39	54	42	1,893	22	3	86	46
0602	Minimal tuberculosis	23	1.89	710	0.2093	38.0	21.8	5.723	0.90	43	102	91	1,420	19	3		
				710	0.1315	34.2	19.8	4.499	0.97	41	51	41	1,299	19	3	68	36
1161	Normal	23	1.82	716	0.2093	51.6	25.5	4.964	0.93	39	109	96	2,284	27	3		
				716	0.1276	59.2	29.5	4.028	1.00	34	57	40	1,966	27	3	73	40
0548	Normal	24	2.03	718	0.2093	49.8	18.0	4.857	0.94	39	109	95	2,132	25	3		
				718	0.1254	66.4	24.0	3.939	1.15	35	59	47	1,884	25	3	75	37
1048	Coin lesion	24	1.93	709	0.2093	60.5	30.5	4.293	1.03	34	115	93	2,066	26	5		
				709	0.1175	67.5	34.5	3.448	1.12	28	58	41	1,709	26	5	68	35
0595	Minimal tuberculosis	26	2.04	709	0.2093	40.4	17.8	5.104	0.80	37	104	92	2,114	22	3		
				709	0.1315	48.3	21.5	4.218	0.98	36	56	44	1,708	22	3	78	38
0819	Coin lesion	27	1.96	714	0.2093	37.2	21.7	5.342	0.88	44	100	86	1,856	22	4		
				714	0.1257	49.9	23.5	4.486	1.02	37	54	41	1,803	22	4	82	42
1101	Coin lesion	35	2.00	718	0.2093	35.4	26.0	4.244	0.84	38	107	86	1,445	22	6		
				718	0.1303	47.2	25.2	3.599	0.86	35	54	41	1,639	22	6	75	38
Mean																74.9	39.7
Standard deviation																6.1	3.4
One Elderly Man, Aged 60 Years																	
0785	Normal	60	1.94	711	0.2093	49.0	29.0	3.664	0.89	37	108	85	1,661	48	4		
				711	0.1619	76.0	32.0	3.146	0.94	31	82	46	1,936	48	4	40	21
One Middle-aged Woman, 37 Years																	
0934	Coin lesion	37	1.74	698	0.2093	26.9	32.0	3.604	0.76	35	102	83	1,028	32	6		
				698	0.1189	39.7	38.5	2.808	1.07	26	59	35	837	32	6	26	15

* No complaint referrable to respiratory system; normal vital capacity. See diagnosis for coin lesion or minimal infiltrate.

Abbreviations:

B.S.A.—body surface area in square meters
BP—barometric pressure
$F_{I_{O_2}}$—concentration of oxygen in inspired air
$\dot{V}$—ventilation in liters per minute
f—respiratory rate
$F_{E_{CO_2}}$—concentration of carbon dioxide in expired air
R—gas exchange ratio
$P_{a_{CO_2}}$—mean arterial blood carbon dioxide tension
$P_{A_{O_2}}$—mean alveolar air oxygen tension
$P_{a_{O_2}}$—mean arterial blood oxygen tension
$\dot{V}_{O_2}$—oxygen consumption per minute

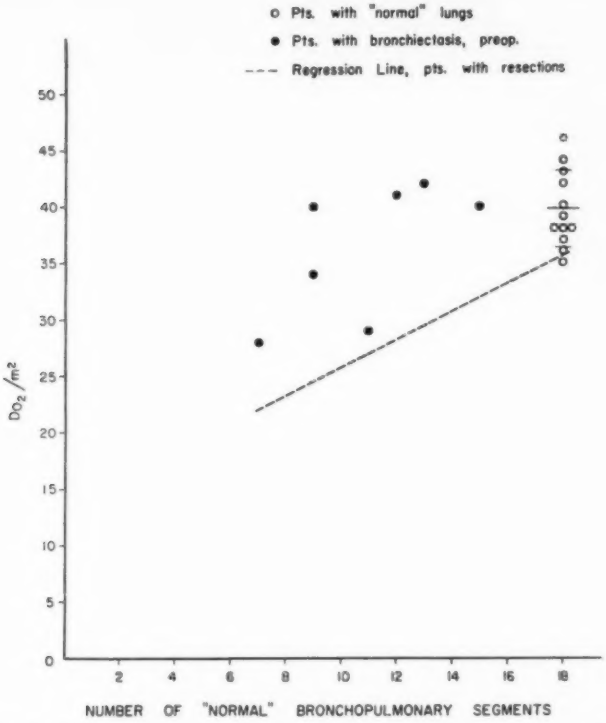

FIG. 1. Diffusing capacity per square meter of body surface area in "normal" subjects and in patients with bronchiectasis. The abscissa is the number of bronchopulmonary segments which, as judged from bronchograms, were free of bronchiectasis. The dotted regression line is the same as the one appearing in figure 2. Horizontal lines in values for "normal" subjects indicate mean and standard deviations.

to the limit of her capacity, had an oxygen consumption of only 837 ml. per min. Her diffusing capacity of 26 or 15 per square meter is next to the lowest recorded in this entire study (*cf.* patient No. 0682, table 5).

Values in Young Men with Bronchiectasis (Table 2)

The diffusing capacities of 7 men with bronchiectasis varied from 50 to 82, the mean and standard deviations being 68.3 ± 12.8. Corresponding figures per square meter of body surface area were 28 to 42 and 36.3 ± 5.9. These 7 patients fell into the same age group as the "normals" and were studied at comparable levels of exercise.

The distribution of diffusing capacities in these normal and bronchiectatic groups is shown in figure 1. It is clear that 4 of the 7 patients with bronchiectasis had normal diffusing capacities, one had a low normal value, and the values in 2 were abnormally low.

Correlation between diffusing capacity and known duration of bronchiectasis, incidence of pneumonia, and amount of sputum was poor. Three of the 4 patients with bronchographic evidence of bilateral basilar bronchiectasis had diffusing capacities which were more than one standard deviation below the mean. There was a poor correlation between diffusing capacity and total lung capacity, volumetric ratios and the maximal breathing capacity. The highest mixing indices and venous admixture ratios, however, appeared in the patients with the lowest diffusing capacities.

The terms "chronic pneumonia" and "atelectasis" consistently appeared in the pathology reports in which the resected specimens were

TABLE 2

CLINICAL, GAS EXCHANGE, AND DIFFUSING CAPACITY DATA OF 7 YOUNG MEN WITH BRONCHIECTASIS*

Clinical Data				Basic Data										Derived Values			
Patient Number	Segments Involved	Age	B.S.A.	BP-47	$F_{I_{O_2}}$	$\dot{V}$	f	$F_{E_{CO_2}}$	R	$P_{E_{CO_2}}$	$P_{A_{O_2}}$	$P_{a_{O_2}}$	$\dot{V}_{O_2}$	$\bar{P}_{A_{O_2}} - \bar{P}_{C_{O_2}}$	Venous Admixture ($\dot{Q}va/\dot{Q}t \times 100$)	Diffusing Capacity	
																Total	M^2
		years	M^2		*mm. Hg*	*l*				*mm. Hg*	*mm. Hg*	*mm. Hg*	*ml.*	*mm. Hg*			
0763	Basilar, left lower lobe (3)	19	1.81	717	0.2093	49.5	51.0	3.832	0.85	40	104	91	1,838	24	4		
				717	0.1257	54.5	37.3	3.926	1.01	37	53	38	1,750	24	4	73	40
0912	Basilar, left lower lobe; lingula (5)	20	1.97	696	0.2093	41.8	20.5	4.999	0.86	43	97	84	1,942	17	4		
				696	0.1243	43.6	22.3	4.264	1.07	43	46	37	1,393	17	4	82	42
0910	Basilar, right lower lobe; right middle lobe (6)	20	1.95	720	0.2093	43.1	25.5	4.393	0.86	44	101	89	1,833	20	4		
				720	0.1250	52.7	25.0	4.242	1.15	39	55	46	1,618	20	4	81	41
0918	Basilar, right lower lobe; basilar, left lower lobe (7)	23	1.90	724	0.2093	52.3	31.0	3.984	0.84	41	104	73	2,079	32	12		
				724	0.1305	78.3	42.0	3.107	1.00	32	62	38	1,792	32	12	56	29
0840	Basilar, right lower lobe; right middle lobe; basilar, left lower lobe (9)	21	1.88	716	0.2093	48.7	23.5	4.443	0.88	40	105	91	2,039	25	4		
				716	0.1279	57.2	26.5	4.121	1.03	37	55	40	1,892	25	4	76	40
0691	Basilar, right lower lobe; basilar, left lower lobe; lingula (9)	22	1.78	705	0.2093	38.4	23.7	4.848	0.90	43	101	81	1,670	26	7		
				705	0.1344	50.2	32.3	3.851	1.00	40	55	36	1,569	26	7	60	34
0795	Basilar, right lower lobe; right middle lobe; basilar, left lower lobe; lingula (11)	26	1.78	700	0.2093	39.9	28.0	4.379	0.90	40	103	83	1,567	28	6		
				700	0.1279	51.3	29.6	3.833	1.15	36	58	38	1,386	28	6	50	28
Mean																68.3	36.3
Standard deviation																12.8	5.9

* Listed according to extent of bronchiectasis as determined from bronchograms.
For abbreviations, *see* table 1.

described. In parts of the lung examined, therefore, there was histologic evidence that the alveolar-capillary membrane had been affected with respect to area, thickness, and composition.

Values in Young Men Studied After Pulmonary Resection (Tables 3 and 4)

Patients studied at level of exercise comparable with that of the "control" group: Thirteen men comparable in age with the patients in the "normal" and "bronchiectatic" groups were studied postoperatively during exercise of equivalent severity. An additional 4 patients, 3 young men and one young woman, were studied postoperatively during less severe exercise. The functional and clinical data of these patients are recorded in tables 3 and 4.

The diffusing capacities of the normal and of the postoperative male patients are plotted in figure 2 as a function of the number of intact

TABLE 3

CLINICAL, GAS EXCHANGE, AND DIFFUSING CAPACITY DATA OF FOUR MEN, AGED 21 TO 35 YEARS, FOLLOWING RESECTION OF AN ISOLATED PULMONARY NODULE*

Clinical Data				Basic Data										Derived Values			
Patient Number	Months Postoperatively	Age	B.S.A.	BP-47	$F_{I_{O_2}}$	$\dot{V}$	f	$F_{E_{CO_2}}$	R	$P_{a_{CO_2}}$	$P_{A_{O_2}}$	$P_{a_{O_2}}$	$\dot{V}_{O_2}$	$\bar{P}_{C_{O_2}} - \bar{P}_{A_{O_2}}$	Venous Admixture ($\dot{V}a/\dot{Q}t \times 100$)	Diffusing Capacity	
																Total	M^2
		years	M^2		*mm. Hg*	*l*				*mm. Hg*	*mm. Hg*	*mm. Hg*	*ml.*	*mm. Hg*			
0815	$2^2/_3$	21	1.87	709	0.2093	40.8	29.0	4.749	0.91	38	107	94	1,741	22	3		
				709	0.1189	53.7	30.0	4.014	1.10	35	52	39	1,605	22	3	73	39
0980	$1^1/_4$	22	1.86	719	0.2093	43.6	31.5	4.606	0.90	37	110	93	1,862	29	4		
				719	0.1314	53.0	38.0	3.683	0.98	34	60	42	1,655	29	4	57	31
1048	$1^3/_4$	24	1.92	713	0.2093	45.7	29.5	4.187	0.87	35	110	97	1,812	30	3		
				713	0.1276	58.1	34.5	3.584	0.98	30	60	40	1,743	30	3	58	30
1101	$0^5/_6$	35	2.00	710	0.2093	51.7	26.0	4.385	0.93	43	103	80	1,994	28	8		
				710	0.1303	64.7	32.0	3.398	0.97	38	54	31	1,850	28	8	66	33
Mean																63.5	33.2

* Uncomplicated postoperative course, vital capacity 83 to 95 per cent of the predicted value. For abbreviations, *see* table 1.

(nonresected) bronchopulmonary segments. Also included in this figure is the diffusing capacity of one patient with a ventilated but unperfused right lung. Examination of this figure and of the pertinent tables reveals the following: (*1*) Patients subjected to resections had subnormal diffusing capacities. No postoperative value was as high as the mean for the "normal" group and all but two of the values differed from the "normal" mean by more than one standard deviation. (*2*) In the group of patients exercised as severely as the normal subjects, the diffusing capacity was proportional to the number of intact or remaining bronchopulmonary segments and inversely proportional to the magnitude of the resection. The equation for the regression of diffusing capacity on the number of intact bronchopulmonary segments was: $y = 13.0 + 1.23\ x$. The correlation coefficient of 0.68 was significant at the 1 per cent level. (*3*) The diffusing capacity of most of the patients possibly was reduced for reasons other than removal of lung tissue per se. This is suggested by the fact that the regression line for the values obtained in patients with resections passes below the mean of the "normal" group, this displacement exceeding one standard deviation. Confidence limits (0.95) on population means of diffusing capacity at $x = 18$ intact bronchopulmonary segments were calculated to be 39.8 and 30.5. The mean for "normal" men was 39.7, a value which falls on the fringe of this confidence belt.

Four of the "normal" patients with isolated pulmonary nodules were studied twenty-five to eighty days following excision of the lesion (table 3). Their postoperative diffusing capacities are plotted in figure 2 as though the patients had had resections of one segment, i.e., over "17" on the abscissa. Their pre- and postoperative studies are compared in figure 3. Although degrees of exercise in the two studies were comparable and although these patients did not have postoperative complications, each of their diffusing capacities was reduced.

Patients studied at level of exercise less than that of "control" group (table 4): Three men and one woman within the same age group as the previous patients were studied at levels of exercise below 1,200 cc. per min. (table 4). Each of the four diffusing capacities of the 3 men fell below the regression line drawn from data on more severely exercising patients (*see* figure 2). One of the four values was below the confidence belt (0.95) for individual values, two were below the confidence belt (0.95) for population means but within the belt for individual samples, and the fourth fell within the confidence belt for both samples and means.

Two diffusing capacity measurements on one 19-year-old girl with resection of eight segments

TABLE 4

CLINICAL, GAS EXCHANGE, AND DIFFUSING CAPACITY DATA OF YOUNG PATIENTS FOLLOWING PULMONARY RESECTION

Clinical Data					Basic Data										Derived Values			
Patient Number	Diagnosis and Magnitude of Resection	Months Postoperatively	Age	B.S.A.	BP-47	$F_{I_{O_2}}$	$\dot{V}$	f	$F_{E_{CO_2}}$	R	Pa_{CO_2}	PA_{O_2}	Pa_O	$\dot{V}_{O_2}$	$\bar{P}_{A_{CO_2}} - \bar{P}_{CO_2}$	Venous Admixture ($\dot{Q}va/\dot{Q}t \times 100$)	Diffusing Capacity	
																	Total	M²
			years	*M²*	*mm. Hg.*		*l*				*mm.Hg.*	*mm.Hg.*	*mm.Hg.*	*ml*	*mm.Hg.*			
Patients with Oxygen Consumption Exceeding 1,300 cc./min.																		
9 Men, Aged 18–31																		
0900	Bronchiectasis		18	1.85	715	0.2093	55.9	36.0	4.394	0.98	38	111	83	2,071	43	8		
	3 segments	2			715	0.1398	79.3	44.7	3.503	1.15	33	71	35	1,993	43	8	46	25
0763	Bronchiectasis		19	1.85	703	0.2093	35.7	39.0	4.082	0.78	40	98	86	1,516	23	4		
	3 segments	3⅕			703	0.1189	48.3	43.5	3.598	1.06	35	50	32	1,329	23	4	58	31
0634	Bronchiectasis		23	1.92	709	0.2093	59.0	32.0	3.899	0.94	40	106	91	2,009	24	4		
	4 segments	2			709	0.1257	60.1	—	3.651	1.20	37	57	44	1,726	24	4	72	37
0684	Bronchiectasis		21	1.83	705	0.2093	41.5	31.0	4.527	0.92	45	100	81	1,649	24	6		
	5 segments	1¾			705	0.1344	51.9	34.0	3.693	1.03	37	58	39	1,512	24	6	63	34
0764	Bronchiectasis		22	1.64	723	0.2093	54.7	43.3	3.437	0.92	31	118	92	1,710	32	5		
	5 segments	3			723	0.1329	62.4	41.3	3.363	1.16	29	71	53	1,515	32	5	47	29
0795	Bronchiectasis		26	1.80	705	0.2093	58.5	37.0	4.147	1.00	41	106	81	1,977		8		
	5 segments				705	0.1365	66.3	46.0	3.151	1.03	32	65	44	1,655	31	8	53	29
		7⅔			705	0.1365	70.9	48.5	3.082	1.04	29	68	44	1,706	34	8	50	28
0840	Bronchiectasis		21	1.89	713	0.2093	42.2	27.0	4.373	0.85	45	98	80	1,799	35	5		
	6 segments	7¼			713	0.1365	52.1	32.0	3.857	1.16	38	64	38	1,444	35	5	41	22
0700	Adenoma		22	2.05	712	0.2093	45.9	27.0	4.628	0.80	38	104	90	2,177	30	4		
	7 segments	1½*			712	0.1302	66.3	32.7	3.663	1.08	34	61	42	1,843	30	4	61	30
0356	Bronchiectasis		31	1.96	719	0.2093	48.1	35.0	4.279	0.96	45	104	76	1,787	37	9		
	10 segments	66*			719	0.1426	62.9	41.3	3.667	1.16	37	70	42	1,650	37	9	45	23

Patients with Oxygen Consumption Less than 1,000 cc./min.

3 Men, Aged 20–26

1366	Cyst, right lower lobe		26	1.86	720	0.2093	27.6	18.0	3.730	0.82	38	106	90	1,047	28	4		
	4 segments	1⅔			720	0.1205	41.2	30.0	3.206	1.12	33	57	38	987	28	4	35	19
0043	Bronchiectasis		20	1.83	727	0.2093	36.3	25.0	3.835	0.95	38	112	96	1,235	23	4		
	7 segments	2½*			727	0.1309	33.1	25.5	3.065	0.92	36	57	43	924	23	4	40	22
0128	Bronchiectasis	11⅔†	20	1.72	713	0.2093	25.7	24.3	4.299	0.89	39	106	89	1,023	23	5		
	6 segments				713	0.1309	29.6	29.0	3.694	1.02	35	59	46	881	23	5	38	22
		4¾*	20	1.72	713	0.2093	29.4	33.0	3.979	0.89	42	103	86	1,083	25	5		
	10 segments				713	0.1261	34.8	32.0	3.376	0.99	37	53	35	975	25	5	39	23

1 Woman, Aged 19

0454	Bronchiectasis		19	1.56	714	0.2093	31.3	35.0	3.205	0.84	34	110	92	985		4		
	8 segments	4¾*			714	0.1315	35.4	39.0	2.712	0.94	28	64	45	840	30	4	28	18
					714	0.1315	32.8	40.5	2.609	0.82	32	56	42	855	25	4	34	22

* Post second operation.
† Post first operation.
For abbreviations, *see* table 1.

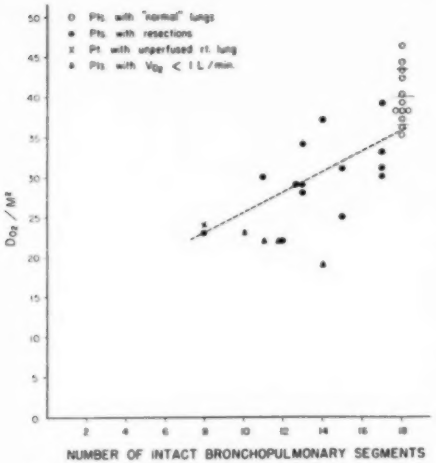
Pts. with "normal" lungs
Pts. with resections
Pt. with unperfused rt. lung
Pts. with V_{O_2} < 1 L/min.
D_{O_2} / M²
NUMBER OF INTACT BRONCHOPULMONARY SEGMENTS

FIG. 2. Diffusing capacity per square meter of body surface area plotted as a function of the number of bronchopulmonary segments in the patient's chest. Regression line calculated from data of patients with V_{O_2} exceeding 1,300 cc. per min. (solid circles) and of patient with unperfused right lung (×). The correlation coefficient of 0.68 was significant at the 1 per cent level.

were 18 and 22. These values are in the same range as those found in young men with comparable resections studied under similar circumstances.

Correlation with other measurements: The per cent of predicted values for the diffusing capacity were correlated with other measurements, the mean of diffusing capacities in the normal group being used as the predicted value. No statistical significance could be attached to the correlation with vital capacity ($r = 0.465$, $P > 0.05$), total lung capacity ($r = 0.398$, $P > 0.05$), or maximal breathing capacity ($r = 0.321$, $P > 0.05$).

Although there was no correlation between magnitude of change in diffusing capacity and in these other parameters, ventilation and lung volumes in these patients were generally abnormal. Only one of the patients included in table 4 had a normal vital capacity (90 per cent of predicted value or more) and only 3 had normal total lung capacities. The residual volume comprised more than 30 per cent of the total lung capacity in 8 patients. In 9 the maximal breathing capacity was less than 70 per cent of the predicted value. One patient had a high mixing index. Each of the

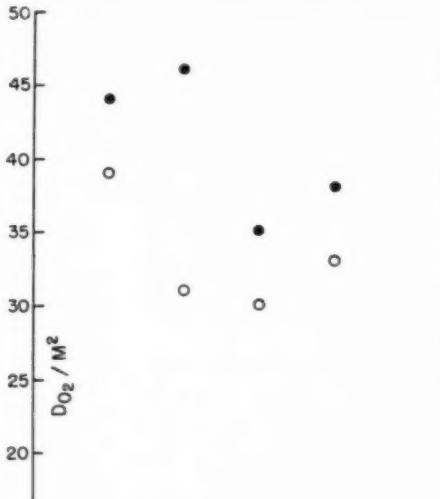

FIG. 3. Diffusing capacity per square meter of body surface area in 4 young men studied before and after resection of an isolated pulmonary nodule. The postoperative diffusing capacity was reduced in each instance.

9 patients for whom bronchospirometry was performed displayed large abnormalities in the distribution of ventilation and oxygen uptake between the two lungs. As a group, therefore, these patients demonstrated not only a reduced diffusing capacity but ventilatory and volumetric abnormalities as well.

Values in Patients with a Variety of Lesions (Table 5)

There were 3 patients with lesions thought to be equivalent to a resection: one young man with minimal right pulmonary arterial blood flow, another with severe obstructive bronchiectasis of the right lower lobe caused by a bronchial ade-

noma, and a 46-year-old man whose right middle lobe was atelectatic following obstruction of its bronchus by lymph nodes. The latter two diagnoses were made in the operating room. The diffusing capacity of each patient was reduced, the magnitude of the reduction in the first 2 corresponding closely to what one would predict from comparable studies of young men plotted on figure 2. Thus, the patient with no right pulmonary artery ("x" in figure 2) and with virtual loss of the capillary bed of 10 bronchopulmonary segments had almost the same diffusing capacity as a patient (No. 0356) with bilateral resections totaling 10 segments. The patient with the obstructed right lower lobe bronchus had a diffusing capacity of 29, a value which, if plotted on figure 2 for a resection of five bronchopulmonary segments, would fall almost on the regression line. Finally, the diffusing capacity of the 46-year-old man with the obstructed right middle lobe (23 per M^2) was comparable with that of the normal 60-year-old man (21 per M^2) listed in table 1.

Four young men *requiring pulmonary decortication* (17) and 2 with *pulmonary cysts* (18) also had low diffusing capacities. The diffusing capacity increased from 39 to 54 in one patient following decortication and from 24 to 38 in another after large blebs had been excised. The contribution of the affected lung to oxygen consumption improved considerably in both instances.

Patient No. 1056 was studied once while his right lung was estimated to be 50 per cent collapsed by a spontaneous pneumothorax and again after this lung had fully re-expanded. Both values for diffusing capacity were between one and two standard deviations below the means for the normal group. Although this patient's vital capacity increased 1.7 liters and his total lung capacity almost 2 liters, his diffusing capacity changed little, the fall from 73 to 68 being equivocal.

Values in Young Men with Disease of the Alveolar Capillary "Membrane" (Table 6)

Diffusing capacity was measured in one patient whose chest roentgenogram was interpreted to indicate pulmonary fibrosis, one patient whose lung biopsy showed interstitial fibrosis, and 5 patients with sarcoid disease of the lungs. In 3 of the latter 5 patients, the diagnosis was based on roentgenographic, clinical, and tissue studies; in the other 2 the roentgenographic and clinical impressions were confirmed by positive serologic tests for syphilis (Kveim). These patients were in the same age group as the "normal" patients and, with the exception of patient No. 1202, were studied at comparable levels of exercise.

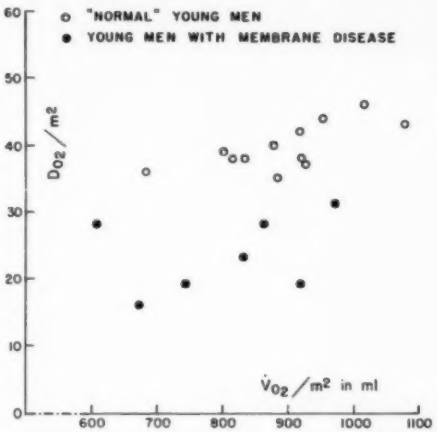

FIG. 4. Diffusing capacity per square meter of body surface area as a function of oxygen consumption per square meter in "normal" young men and in young men with disease of the alveolar capillary "membrane." The diffusing capacities of the latter were reduced at all metabolic levels studied.

The mean of the diffusing capacities per square meter of body surface area was 23.4 ± 5.7 as compared with 39.7 ± 3.4 for the "normal" group. No patient had a diffusing capacity higher than two standard deviations below the normal mean. These differences between the two groups are shown in figure 4, in which the diffusing capacities are plotted as a function of metabolic rate. It is clear from this figure that the diffusing capacity was lower in the group of patients with membrane disease at all levels of oxygen consumption for which values in normal young men were obtained (from approximately 680 ml. per min. per M^2 to 1,080 ml. per min. per M^2).

DISCUSSION

The present studies are consistent with the hypothesis that values obtained for pulmonary

TABLE 5

CLINICAL, GAS EXCHANGE, AND DIFFUSING CAPACITY DATA OF PATIENTS WITH A VARIETY OF LESIONS

Clinical Data				Basic Data										Derived Values			
Patient Number	Diagnosis and Operative Status	Age	B.S.A.	BP-47	$F_{I_{O_2}}$	$\dot{V}$	f	$F_{E_{CO_2}}$	R	Pa_{CO_2}	$P_{A_{O_2}}$	Pa_{O_2}	$\dot{V}_{O_2}$	$\bar{V}_{A_{O_2}}-P_{CO_2}$	Venous Admixture($\dot{Q}va/\dot{Q}t \times 100$)	Diffusing Capacity	
																Total	M^2
		years	M^2	*mm. Hg.*		*l*				*mm. Hg.*	*mm. Hg.*	*mm. Hg.*	*ml.*	*mm. Hg.*			
Patients with Lesions Equivalent to a Resection																	
0929	Absence of right pulmonary artery	25	1.95	709	0.2093	46.7	28.0	4.364	0.90	36	109	85	1,848	35	7		
	Preoperative			709	0.1426	63.8	34.0	3.474	1.11	34	70	45	1,638	35	7	47	24
0608	Obstructive bronchiectasis, right lower lobe	20	2.02	714	0.2093	36.0	22.5	4.902	0.87	44	100	96	1,687	24	1		
	Preoperative			714	0.1306	43.6	29.0	3.769	0.96	35	57	45	1,419	24	1	59	29
0857	Atelectasis, right middle lobe	46	1.92	714	0.2093	45.9	26.0	4.194	0.89	39	107	80	1,776	36	8		
	Preoperative			714	0.1426	60.3	29.5	3.413	1.04	31	72	47	1,623	36	8	45	23
Patients Requiring Pulmonary Decortication																	
0616*	Tuberculous empyema	22	2.03	711	0.2093	46.7	32.3	4.172	0.89	40	105	102	1,800	24	1		
	Preoperative			711	0.1306	69.1	—	3.115	0.98	35	57	44	1,806	24	1	75	37
	2½ months after left decortication		2.04	712	0.2093	54.1	31.5	4.313	0.92	36	111	87	2,077	28	6		
				712	0.1261	82.7	40.0	3.718	1.13	33	60	42	2,237	28	6	80	39
0743	Pleurisy, idiopathic	24	1.92	713	0.2093	39.8	21.0	4.647	0.88	41	104	80	1,732	25	8		
	Preoperative			713	0.1257	61.6	30.7	3.704	1.07	37	55	37	1,750	25	8	70	36
	3 weeks after left decortication		1.94	709	0.2093	44.1	28.0	4.423	0.92	39	107	86	1,734	28	6		
				709	0.1254	62.0	33.0	3.784	1.22	38	57	36	1,567	28	6	78	40
0702*	Empyema, tuberculous, left; pulmonary tuberculosis, moderately advanced, right upper lobe	23	1.78	726	0.2093	35.7	26.0	3.880	0.88	42	105	73	1,326	31	12		
				726	0.1279	50.7	34.5	3.011	1.06	33	61	37	1,209	31	12	39	22
			1.78	721	0.2093	44.5	22.0	3.956	0.81	37	107	81	1,802	27	8		
	Preoperative 4 months after left decortication			721	0.1175	64.7	33.0	2.771	1.02	30	55	35	1,470	27	8	54	31
0668	Empyema, tuberculous, right	29	1.63	716	0.2093	60.6	—	2.331	0.94	18	131	83	1,241	29	10		
	Preoperative			716	0.1261	55.2	—	1.925	0.94	22	67	48	938	29	10	32	20

Patients with Pulmonary Cysts (Blebs)																	
0682†	Both upper lobes	31	1.80	717	0.2093	44.0	37.0	3.668	0.98	28	122	58	1,362	45	26		
	Preoperative			717	0.1426	47.8	37.0	2.973	1.10	29	76	34	1,065	45	26	24	13
	1½ months after large cysts removed from right upper lobe		1.80	711	0.2093	42.1	34.7	3.600	0.90	39	107	83	1,376	31	7		
				711	0.1344	49.3	37.0	3.141	1.07	36	62	40	1,186	31	7	38	21
	2¾ months after small cysts removed from left upper lobe and left lower lobe (wedge resection)		1.82	703	0.2093	40.0	34.5	3.770	0.85	40	102	83	1,442	30	6		
				703	0.1305	55.6	41.7	3.164	1.17	36	60	39	1,224	30	6	41	23
0784	Bilateral, upper lobes	33	1.95	713	0.2093	50.8	22.7	4.348	0.95	40	108	80	1,904	31	8		
	Preoperative			713	0.1257	72.0	30.0	2.944	1.05	30	61	38	1,658	31	8	53	27
Patient with a Spontaneous Pneumothorax																	
1056	50% collapse of right lung	28	2.08	718	0.2093	86.0	34.9	3.478	0.98	32	118	83	2,527	33	8		
				718	0.1398	75.7	27.3	3.499	0.91	30	68	45	2,407	33	8	73	35
	3 weeks later; re-expanded entirely			724	0.2093	77.1	25.0	4.184	0.98	32	119	92	2,739	32	6		
				724	0.1314	80.2	26.0	3.502	1.08	31	66	46	2,170	32	6	68	33

* Data included in reference 17
† Data included in reference 18
For abbreviations, *see* table 1.

TABLE 6

CLINICAL, GAS EXCHANGE, AND DIFFUSING CAPACITY DATA OF SEVEN YOUNG MEN WITH DISEASE OF ALVEOLAR-CAPILLARY "MEMBRANE"

Clinical Data					Basic Data										Derived Values			
Patient Number	Diagnosis	Basis of Diagnosis	Age	B.S.A.	BP-47	F_{H_2}	$\dot{V}$	f	F_{ECO_2}	R	Pa_{CO_2}	$P_{A_{O_2}}$	Pa_{O_2}	$\dot{V}_{O_2}$	$\bar{P}_{A_{O_2}} - P_{CO_2}$	Venous Admixture $\left(\frac{\dot{Q}ya}{\dot{Q}t} \times 100\right)$	Diffusing Capacity	
																	Total	M^2
			yrs.	M^2	*mm. Hg.*		*l*				*mm. Hg.*	*mm.Hg*	*mm.Hg*	*ml.*	*mm.Hg.*			
1017	Sarcoidosis	Roentgenogram	22	1.96	715	0.2093	46.3	20.5	5.270	0.94	43	105	84	2,135	31	6		
		Kveim test			715	0.1368	55.7	23.5	4.168	1.13	38	64	43	1,696	31	6	55	28
1059	Sarcoidosis	Cervical node biopsy	22	1.97	710	0.2942	38.4	31.0	5.580	0.87	53	150	93	2,022	49	9		
					710	0.1619	63.8	46.5	3.830	1.10	41	77	34	1,813	49	9	37	19
1202	Sarcoidosis	Epididymis biopsy	21	1.81	713	0.2093	40.1	—	3.577	0.81	41	100	94	1,469	22	2		
					713	0.1185	41.6	—	3.177	0.99	32	52	39	1,105	22	2	50	28
1272	Pulmonary fibrosis	Roentgenogram only	26	1.91	707	0.2093	46.7	26.0	3.688	0.88	30	115	80	1,606	36	9		
					707	0.1276	70.6	29.5	2.924	1.06	23	68	41	1,591	36	9	44	23
1334	Sarcoidosis	Lung biopsy	22	1.85	700	0.2093	47.5	30.5	3.997	0.94	35	110	83	1,636	32	8		
					700	0.1346	65.2	38.0	3.979	1.13	31	66	45	1,858	32	8	58	31
1360	Interstitial fibrosis	Lung biopsy	27	1.82	708	0.2093	42.8	35.5	3.488	0.87	43	100	55	1,407	41	24		
					708	0.1619	47.9	37.0	3.200	1.02	43	72	35	1,229	41	24	30	16
1425	Sarcoidosis	Roentgenogram	24	2.13	717	0.2093	—	—	3.483	0.83	44	99	75	—	39	7		
		Kveim test			717	0.1346	67.5	—	2.836	1.00	30	67	33	1,587	39	7	41	19
Mean																	45.0	23.4
Standard deviation																	±10.0	±5.7

For abbreviations, *see* table 1.

diffusing capacity for oxygen are affected by the area and permeability of the alveolar-capillary "membrane." Sensitivity of the method to alterations in area is suggested by: (*a*) the decrease in diffusing capacity associated with a decrease in the number of bronchopulmonary segments remaining after pulmonary resection; (*b*) the low diffusing capacities found in patients with preoperative lesions which probably affect principally the area of the alveolar-capillary bed (absence of right pulmonary arterial blood flow, atelectasis, severe bronchial stenosis, empyema); and (*c*) the increase in diffusing capacity demonstrated by patients whose roentgenograms and bronchospirometry indicated expansion of previously restricted lung. Sensitivity to lesions which classically affect primarily thickness and composition of the "membrane" is shown by the low diffusing capacities of patients with sarcoid disease or interstitial fibrosis of the lung. In summary, these two observations bear out the validity of this method as a means of assessing characteristics of the alveolar-capillary "membrane."

The plausibility of some of the interpretations placed on diffusing capacity data is supported by these observations. The increase in diffusing capacity during exercise, for example, has been interpreted to indicate the opening of additional pulmonary capillaries (15). Reduction of diffusing capacity with age (14) and in some patients with obstructive disease (19) has been thought to reflect attenuation of the alveolar-capillary bed. The high alveolar-arterial gradients and low diffusing capacities in the "alveolar-capillary block" group of diseases have been attributed to changes in membrane permeability (20).

These observations also support the concept that diffusing capacity reaches maximal values. Riley and associates (1), have found that the increase in diffusing capacity associated with exercise is limited to levels of oxygen consumption below approximately 1,200 cc. per min.; above these metabolic rates "maximal diffusing capacities" were reached, suggesting maximal expansion of the pulmonary capillary network. The fact that diffusing capacity was depressed at metabolic rates exceeding 1,200 cc. per min. in the patients with resections supports the concept that there is a maximal diffusing capacity. Were there no limit to the number of latently perfused capillaries surrounding each alveolus, the maximal dimensions of the alveolar-capillary bed would be independent of the number of alveoli, and one would not anticipate decreasing these dimensions by excising lung tissue. To the extent that diffusing capacity in the absence of permeability change reflects primarily membrane area, these studies suggest that resection, by removing alveoli and their capillaries, imposes a lower limit to maximal alveolar-capillary surface area.

The adjective "maximal" deserves special emphasis, for it may be that the "minimal" alveolar-capillary dimensions of resting subjects are not reduced by excision of lung tissue. Cournand and co-workers (3), for example, reported normal diffusing capacities in patients following pneumonectomy; the studies were, however, performed at low metabolic levels. If one accepts these values as well as the present ones, one is led to conclude: that expansion of the alveolar-capillary bed relative to the number of alveoli is one of the adjustments to an area-reducing lesion, and that this compensation is limited in its scope. Viewed in this light, the alveolar-capillary bed of the resting patient who has had a resection has encroached on the expansion of which this bed was capable preoperatively during exercise.

The diffusing capacities listed in table 1 for "normal" subjects are higher than those previously reported for comparable rates of oxygen consumption. The highest value tabulated by Cohn and associates (14) in subjects within the same age limits was 80, or 38 per square meter of body surface area, as compared with 86 (46 per square meter) in one of the subjects in the present study. There were 13 values below 35 per M^2 among the 19 reported by Cohn and associates; there were none among the patients reported by the present writers. If one excludes analytic errors, these differences might be explained by the greater oxygen consumptions and the younger ages of the patients in the present study. Whereas 11 of their measurements were obtained at metabolic rates less than 850 cc. per min. per M^2, this was true in only 4 of 12 in the present series; the mean oxygen consumption among their patients was 858 per M^2 as compared with 912 per M^2 among those in the present report. Six of their nine subjects between seventeen and thirty-five years of age were twenty-nine years or more; 11 of the 12 subjects in the present study were twenty-seven years old or less (*see* table 1). Since diffusing capacity decreases with

metabolic rate and age, these differences might account for the higher values in the present series. It may also be that there is a relationship between diffusing capacity and physical fitness; the subjects in this series, being on active duty in the armed forces, may have differed from those of Cohn and associates in this respect.

A vigorous 60-year-old man (patient No. 0785) had a diffusing capacity approximately one-half that of the younger subjects. A 46-year-old man (patient No. 0857) with atelectasis of the right middle lobe had a diffusing capacity almost identical with that of a 25-year-old subject whose entire right lung was virtually unperfused (patient No. 0929). In neither of these patients could poor exercise tolerance account for the low values. Reduction in the maximal area of the alveolar-capillary bed with age appeared to be a reasonable explanation (14).

The two women included in this study (patient No. 0934, table 1, and patient No. 0454, table 4) achieved metabolic rates substantially below those reached by the men. Mean gradients, however, ranged from 25 to 32, values at which maximal diffusing capacities are usually obtained (1). In one of these, a 37-year-old officer with a "coin" lesion, the diffusing capacity was 15 per M^2, considerably less than in the man of sixty. The other woman was eighteen years younger than the previous one and gave the impression of having greater exercise tolerance despite her extensive disease. The mean of her two diffusing capacities was 20 per M^2. This is nearly the same figure as that recorded for a young man (patient No. 0128) who was comparable with respect to age, magnitude of resection, and level of exercise. The first study, then, suggests that there is a sex difference in diffusing capacity; the second does not clearly confirm this observation.

The four postoperative diffusing capacities (figure 2) measured at metabolic rates below 1,200 cc. of oxygen per minute fell below the regression line calculated from studies performed at higher rates. Rigid statistical criteria for considering these values significantly different from the others were present in only one of the 4 instances. However, the fact that each is lower than would be expected from regression is compatible with the observation that diffusing capacity increases with the severity of exercise at rates of oxygen uptake below 1,200 cc. per min. (15).

That the effect of bronchiectasis on diffusing capacity is variable is shown in figure 1. Williams (21) has suggested that patients in whom a fall is found also have emphysema and that the low diffusing capacity in these results from the latter. The changes in permeability and area implied in the anatomic studies of lung tissue resected from the patients with bronchiectasis render this hypothesis unnecessary without eliminating it. Moreover, 2 of the 3 patients with clearly depressed diffusing capacities had normal maximal breathing capacities and normal residual volumes/total lung capacity ratios; emphysema in these was unlikely. One can conclude that the diffusing capacity may or may not be depressed in patients with bronchiectasis and that, when it is depressed, emphysema need not be present.

It may be seen in figure 2 that maximal diffusing capacity is reduced in proportion to magnitude of resection. One possible source of error in arriving at this conclusion requires special mention, namely, the relative brevity of the convalescent period in most postoperative patients and the effect of surgical trauma on the diffusing capacity of the remaining lung. Recovery from the effects of pulmonary surgery on lung volumes and ventilation continues after six months (22, 23). Most of the patients were studied less than four months postoperatively. Why did the diffusing capacity of each patient with localized excision of an isolated pulmonary nodule decrease postoperatively? Is trauma to the lung proportional to magnitude of resection and is the slope of the regression line in figure 2 a function of incomplete "recovery" of remaining lung rather than of the estimated amount of remaining lung?

Although there are no certain replies to these questions, the study of patient No. 0356 indicates that diffusing capacity may be reduced in proportion to resection even when convalescence has been prolonged. This patient had bilateral resections and was studied five and a half years postoperatively; his diffusing capacity was about what one would predict from estimating the amount of lung tissue remaining.

Whether the slope of the regression line in figure 2 is attributable to area reduction caused by excision of lung tissue or whether it is attributable to undetermined causes such as trauma, other data show that area reduction is itself associated with a lowered diffusing capacity. Thus, a low diffusing capacity was found in patients (table 5) who had no operation but who had lesions thought to affect primarily the area of the alveolar-capillary bed; when there was a

basis for predicting how low the diffusing capacity might be, there was close agreement between the prediction and the value derived from figure 2. Moreover, the diffusing capacities of exercising dogs (24) are approximately what one would expect if diffusing capacity is a function of membrane area and if, as seems reasonable, membrane permeabilities in man and dog are comparable and membrane areas differ in proportion to body weight.

In summary, it is conceivable that trauma to the lung and brevity of convalescence have somehow affected some of the measurements. In the absence of such effects, the regression line in figure 2 would probably be steeper and pass closer to the mean of normal values.

Diffusing capacity, measured by a number of techniques, has been shown to be low in patients with disease of the alveolar-capillary "membrane." In studies in which diffusing capacity for oxygen was measured directly, however, the levels of exercise were low (25–28). There is the possibility, therefore, that the low figures reflect poor exercise tolerance and incomplete expansion of the alveolar-capillary bed rather than the effects of the lesion itself. The data of figure 4, as well as the studies of Cugell and associates (29) with carbon monoxide, show that low diffusing capacity may be found in patients with "membrane disease" who nevertheless achieve relatively nigh metabolic rates.

Pulmonary fibrosis may (30) or may not (31) be associated with obstructive disease and "hyperinflation." In the latter instance, diffusing capacity might be reduced for reasons similar to those sited for emphysema (19). As a group, however, the patients in the present series had normal maximal breathing capacities, somewhat reduced total lung capacities, normal volumetric ratios (one exception), and normal mixing indices (one exception). With one possible exception, therefore, the characteristics of obstructive disease were lacking. A likely explanation for the low diffusing capacities appeared to be abnormality of the alveolar walls themselves.

The low diffusing capacities among the patients with "membrane disease" may not, of course, be attributable exclusively to changes in permeability, for "membrane disease" is commonly compounded by reductions in "membrane" area; not only do the alveolar walls increase in thickness and change in composition, but alveoli themselves are entirely replaced by the abnormal tissue (32, 33). It is improbable, therefore, that "membrane disease," in the sense of permeability change independent of area reduction, appeared among the patients listed in table 4.

The data were examined to see if the ventilatory equivalent,[6] a crude index of the economy of gas exchange, revealed differences between normal and abnormal groups of patients. This was done by computing ventilatory equivalents during exercise at both levels of oxygenation in normal subjects, in the 9 patients with resections included in table 4, and in 6 of the 7 patients with "membrane disease" included in table 6. It was found that the increase in ventilatory equivalent with hypoxia was significantly greater (P 0.01) in the abnormal than in the normal group (1.15 ± 0.26 abnormal, 0.74 ± 0.50 normal). Such a comparison is meaningful, of course, only if metabolic rates and degrees of hypoxia are comparable, as, indeed, they were. The necessity for creating high gradients by maintaining higher-than-normal alveolar oxygen tensions did, therefore, impose on these patients an additional metabolic cost of breathing.

Two other factors contributing to the inefficiency of respiration in such patients are an increase in physiologic dead space (4, 25) and a decreased compliance (34, 36). Both of these changes signify augmented work of breathing at any level of alveolar ventilation.

Inevitably, therefore, and quite apart from factors relating to airway resistance, reduced diffusing capacity attributable either to reduced area or decreased permeability must be associated under some circumstances with increased respiratory work and effort.

Summary

The method of Lillienthal, Riley, Proemmel, and Franke was used for measuring the pulmonary diffusing capacity for oxygen in the following groups: 12 "*normal*" young men with isolated pulmonary nodules ("coin" lesions) or with no demonstrated pulmonary pathology, 7 young men with *bronchiectasis*, 4 young men following resection of an isolated pulmonary nodule, 12 young men following *resections* varying in magnitude from 3 to 10 bronchopulmonary segments, 10 young men with a *variety of lesions* affecting primarily the area of the alveolar-capillary mem-

[6] Ventilation in liters per 100 ml. oxygen consumption.

brane, and 7 young men with *disease of the alveolar-capillary membrane*. Observations bearing on the effects of age, sex, and severity of exercise were also made. In most studies, metabolic rates at the lower level of oxygenation exceeded 1,200 cc. per minute, end-gradients were 12 mm. of mercury or more, and mean gradients were 22 mm. of mercury or more.

"*Normal*" values were 39.7 ± 3.4 per square meter of body surface area.

Bronchiectasis was associated with a low diffusing capacity in 3 of the 7 patients; each of these had involvement of the basilar segments of both lower lobes.

Among the patients with *resections*, the diffusing capacity was proportional to the number of intact or remaining bronchopulmonary segments. The diffusing capacity was reduced twenty-five to eighty-one days following resection of a "coin" lesion.

Diffusing capacities among the patients with a *variety of lesions* were reduced when the area of the functioning alveolar-capillary membrane was probably decreased, and were increased following surgical procedures thought to result in an enlarged diffusing surface.

Each of the 7 men with "*membrane*" *disease* (sarcoidosis, interstitial fibrosis, pulmonary fibrosis) had a low diffusing capacity, the mean for the group being 23.4 as contrasted to 39.7 in the "normal" group.

The correlation between diffusing capacity and other parameters of function was generally poor.

These data bear out the hypothesis that the method of Lillienthal, Riley, Proemmel, and Franke for measuring diffusing capacity is affected by changes in area of the alveolar-capillary membrane as well as by changes in its permeability.

Sumario

Mediciones de la Capacidad Difusora del Pulmón para el Oxígeno durante el Ejercicio

El método de Lillienthal, Riley, Proemmel y Franke fué usado para medir la capacidad difusora del pulmón para el oxígeno en los siguientes grupos: 12 jóvenes "normales" con nódulos pulmonares aislados (lesiones redondas) o con patología pulmonar reconocida, 7 jóvenes con *bronquiectasia*, 4 jóvenes consecutivamente a *resección* de un nódulo pulmonar aislado, 12 jóvenes consecutivamente a *resecciones* cuya magnitud variaba de 3 a 10 segmentos broncopulmonares, 10 jóvenes con *lesiones variadas* que afectaban primariamente la zona de la membrana alvéolo-capilar y 7 jóvenes con *enfermedad de la membrana alvéolo-capilar*. También se hicieron observaciones relacionadas con los efectos de la edad, del sexo y de la intensidad del ejercicio. En la mayor parte de los estudios, los coeficientes metabólicos al nivel menor de oxigenación excedían de 1,200 c.c. por minuto y las pendientes finales eran de 12 mm. de mercurio o más y las medias de 22 mm. de mercurio o más.

Los valores "*normales*" fueron de 39.7 ± 3.4 por metro cuadrado de área del cuerpo.

La *bronquiectasia* se asoció con baja capacidad difusora en 3 de los 7 enfermos, cada uno de los cuales tenía afectados los segmentos basilares de ambos lóbulos inferiores.

Entre los enfermos con *resecciones*, la capacidad difusora guardó relación con el número de segmentos broncopulmonares intactos o restantes. Se redujo dicha capacidad a los veinticinco a ochenta y un días de la resección de una lesión redonda.

Entre los enfermos con *lesiones variadas*, se rebajó la capacidad difusora cuando estaba probablemente disminuída el área funcionante de la membrana alvéolo-capilar y se elevó a continuación de procedimientos quirúrgicos a los que se atribuía un aumento de la superficie difusora.

Cada uno de los 7 sujetos que tenían "*enfermedad de la membrana*" (sarcoidosis, fibrosis intersticial, fibrosis pulmonar) mostró baja capacidad difusora, siendo el promedio para el grupo de 23.4, en contraposición a 39.7 para el grupo normal.

La correlación entre la capacidad difusora y otros parámetros de la función fué en general mala.

Confirman estos datos la hipótesis de que el método de Lillienthal, Riley, Proemmel y Franke para medir la capacidad difusora se ve afectado por las alteraciones del área de la membrana alvéolo-capilar a la par que por las alteraciones de su permeabilidad.

Resume

Mesures de la capacité pulmonaire de diffusion de l'oxygène au cours de l'exercice

La méthode de Lillienthal, Riley, Proemmel et Franke a été employée pour mesurer la capacité pulmonaire de diffusion de l'oxygène dans les groupes suivants: 12 jeunes gens "normaux", présentant des nodules pulmonaires isolés (lésions en "pièce de monnaie"), ou sans lésion pulmonaire prouvée, 7 jeunes gens atteints de *bron-*

ehectasie, 4 ayant subi une résection d'un nodule pulmonaire isolé, 12 ayant subi des résections dont l'importance variait de 3 à 10 segments broncho-pulmonaires, 10 présentant *diverses lésions*, affectant surtout la paroi alvéolo-capillaire, et 7 présentant une *affection de la paroi alvéolo-capillaire*. Les effets de l'âge, du sexe et de la rigueur de l'exercice furent aussi observés. Dans la plupart des cas, les taux du métabolisme au niveau minimum d'oxygénation ont dépassé 1.200 cc par minute, le niveau limite étant de 12 mm de mercure ou plus et le niveau moyen de 22 mm de mercure ou plus.

Les mesures "*normales*" étaient de 39,7 ± 3,4 par m² de surface du corps.

La *bronchectasie* coincidait avec une capacité de diffusion basse chez 3 des 7 malades; chacun d'eux présentait une atteinte des segments basilaires des deux lobes inférieurs.

Chez les malades ayant subi une *résection*, la capacité de diffusion était proportionnelle au nombre restant des segments broncho-pulmonaires indemnes. La capacité de diffusion était réduite dans les ving-cinq à quatre-ving-et-un jours suivant la résection de la lésion en "pièce de monnaie".

La capacité de diffusion était réduite chez les malades présentant des *lésions diverses* quand la zône où la paroi alvéolo-capillaire fonctionnait était probablement diminuée, et elle était augmentée à la suite d'interventions chirurgicales destinées à augmenter la surface de diffusion.

Chacun des 7 hommes atteints d'affection de la *paroi alvéolo-capillaire* (sarcoidose, sclérose interstitielle, sclérose pulmonaire) présentait une diminution de la capacité de diffusion, la moyenne de cette capacité étant 23,4 pour ce groupe, comparée à 39,7 pour le groupe "normal".

La corrélation entre la capacité de diffusion et les autres paramètres de la fonction pulmonaire était généralement pauvre.

Ces résultats confirment l'hypothèse que la méthode de Lillienthal, Riley, Proemmel et Franke, pour mesurer la capacité de diffusion est influencée par les modifications de la surface de la paroi alvéolo-capillaire aussi bien que par les modifications de sa perméabilité.

Acknowledgments

The authors are indebted to Dr. J. Mead, Dr. B. G. Ferris, Jr., and Dr. E. D. Robin for their helpful criticism of the manuscript, and to Dr. R. H. Shepard for counsel at the time this study was begun in 1952.

The technical assistance of the following hospital corpsmen of the United States Navy is gratefully acknowledged: Silverio Almeida, Jerry Borrelli, John Briggs, Henry Haddad, Donald Lieswald, Robert McGreevy, Sanford Miller, Richard Monsees, Robert Morgan, Robert Obser, Ernest Picknally, David Robinson, and Richard Strutz.

REFERENCES

(1) Riley, R. L., Shepard, R. H., Cohn, J. E., Carroll, D. C., and Armstrong, B. W.: Maximal diffusing capacity of lungs, J. Appl. Physiol., 1954, *6*, 573.

(2) Comroe, J. H., Jr., Forster, R. E., Dubois, A. B., Briscoe, W. A., and Carlsen, E., The Lung, Year Book Publishers, Chicago, Ill., 1955.

(3) Cournand, A., Riley, R. L., Himmelstein, A., and Austrian, R.: Pulmonary circulation and alveolar ventilation-perfusion relationships after pneumonectomy, J. Thoracic Surg., 1954, *19*, 80.

(4) Siebens, A. A., Smith, R. E., and Storey, C. F.: Physiological changes attending bilateral pulmonary resection, Surg. Forum, 1953, *4*, 238.

(5) Lillienthal, J. L., Riley, R. L., Proemmel, D. D., and Franke, R. E.: An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude, Am. J. Physiol., 1946, *147*, 199.

(6) Riley, R. L., and Cournand, A.: Analysis of factors affecting partial pressures of O_2 and CO_2 in gas and blood of lungs: Theory, J. Appl. Physiol., 1951, *4*, 77.

(7) Riley, R. L., Cournand, A., and Donald, K. W.: Analysis of factors affecting partial pressures of O_2 and CO_2 in gas and blood of lungs: Methods, J. Appl. Physiol., 1951, *4*, 102.

(8) Riley, R. L., Proemmel, D. D., and Franke, R. E.: Direct method for determination of oxygen and carbon dioxide tensions in blood, J. Biol. Chem., 1945, *161*, 621.

(9) Riley, R. L., Campbell, E. J. M., and Shepard, R. H.: A bubble method for estimation of PCO_2 and PO_2 in whole blood, J. Appl. Physiol., 1957, *11*, 245.

(10) Scholander, P. F.: Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples, J. Biol. Chem., 1947, *167*, 1.

(11) Van Slyke, D. D., and Neill, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement, J. Biol. Chem., 1924, *61*, 523.

(12) Darling, R. C., Cournand, A., and Richards, D. W., Jr.: Studies on intrapulmonary mixture of gases: Open circuit method for measuring residual air, J. Clin. Invest., 1940, *19*, 609.

(13) Baldwin, E. deF., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: Physiological classification, clinical meth-

ods of analysis, standard values in normal subjects, Medicine, 1948, *27*, 243.

(14) Cohn, J. E., Carroll, D. G., Armstrong, B. W., Shepard, R. H., and Riley, R. L.: Maximal diffusing capacity of lung in normal male subjects of different ages, J. Appl. Physiol., 1954, *6*, 588.

(15) Forster, R. E.: Exchange of gases between alveolar air and pulmonary capillary blood, Physiol. Rev., 1957, *37*, 391.

(16) Pappenheimer, J. R.: Standardization of definitions and symbols in respiratory physiology, Fed. Proc., 1950, *9*, 602.

(17) Siebens, A. A., Storey, C. F., Newman, M. M., Kent, D. C., and Standard, J. E.: The physiological effects of fibrothorax and the functional results of surgical treatment, J. Thoracic Surg., 1956, *32*, 53.

(18) Siebens, A. A., Grant, A. R., Kent, D. C., Klopstock, R., and Cincotti, J. J.: Pulmonary cystic disease: Physiologic studies and results of resection, J. Thoracic Surg., 1957, *33*, 185.

(19) Shepard, R. H., Cohn, J. E., Cohen, G., Armstrong, B. W., Carroll, D. G., Donoso, H., and Riley, R. L.: The maximal diffusing capacity of the lung in chronic obstructive disease of the airways, Am. Rev. Tubere., 1955, *71*, 249.

(20) Austrian, R., McClement, J. H., Renzetti, A. D., Jr., Donald, K. W., Riley, R. L., and Cournand, A.: Clinical and physiologic features of some types of pulmonary diseases with impairment of alveolar-capillary diffusion, Am. J. Med., 1951, *11*, 667.

(21) Williams, M. H., Jr.: Pulmonary function in bronchiectasis, Army Medical Service Graduate School Project 6-60-13-017, Subtask No. 13, 1954.

(22) Kamener, R., Becklake, M. R., Goldman, H., and McGregor, M.: Respiratory function following segmental resection of the lung for bronchiectasis, Am. Rev. Tubere., 1958, *77*, 209.

(23) Smith, G. A., Siebens, A. A., and Storey, C. F.: Preoperative and postoperative cardiopulmonary function studies in patients with bronchiectasis, Am. Rev. Tubere., 1954, *69*, 869.

(24) Siebens, A. A., Rauf, R. A., and Ray, L.: Diffusing capacity of dog's lungs, The Physiologist, 1957, *1*, 78.

(25) Riley, R. L., Riley, M. C., and Hill, H. M.: Diffuse pulmonary sarcoidosis, Bull. Johns Hopkins Hosp., 1952, *91*, 345.

(26) Stone, D. J., Schwartz, A., Feltman, J. A., and Lovelock, F. J.: Pulmonary function in sarcoidosis, Am. J. Med., 1953, *15*, 468.

(27) Marks, A., Cugell, D. W., Cadigan, J. B., and Gaensler, E. A.: Clinical determination of diffusion capacity of lungs, Am. J. Med., 1957, *22*, 51.

(28) West, J. R., McClement, J., Carroll, D. G., Bliss, H. A., Kuschner, M., Richards, D. W., Jr., and Cournand, A.: Effects of cortisone and ACTH in cases of chronic pulmonary disease with impairment of alveolar-capillary diffusion, Am. J. Med., 1951, *10*, 156.

(29) Cugell, D. W., Marks, A., Ellicott, M. F., Badger, T. L., and Gaensler, E. A.: Carbon monoxide diffusing capacity during steady exercise, Am. Rev. Tubere., 1956, *74*, 317.

(30) Coates, E. O., and Comroe, J. H.: Pulmonary function studies in sarcoidosis, J. Clin. Invest., 1951, *30*, 848.

(31) Baldwin, E. deF., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: II. A study of 39 cases of pulmonary fibrosis, Medicine, 1949, *28*, 1.

(32) Longcope, W. T., and Freiman, D. G.: Study of sarcoidosis, Medicine, 1952, *31*, 1.

(33) Hamman, L., and Rich, A. R.: Acute diffuse interstitial fibrosis of the lungs, Bull. Johns Hopkins Hosp., 1944, *74*, 177.

(34) Lyons, H. A.: Pulmonary compliance in granulomatous disease of the lung, Am. J. Med., 1958, *25*, 23.

(35) McIlroy, M. B., and Bates, D. V.: Respiratory function after pneumonectomy, Thorax, 1956, *11*, 303.

(36) Gliedman, M. L., Siebens, A. A., Timmes, J. J., Pino, D. M., Vestal, B. L., and Karlson, K. E.: Unilateral lung compliance during thoracotomy, Ann. Surg. (in press).

(37) Siebens, A. A., and Frank, N. R.: Diffusing capacity at exercise in patients with alterations in area of the pulmonary capillary bed, Am. J. Physiol., 1954, *179*, 673.

(38) Siebens, A. A., Frank, N. R., Rauf, R. A., and Ray, L.: Diffusing capacity and area of alveolar capillary bed, Fed. Proc., 1957, *16*, 119.

OBJECTIVE LONG-TERM EVALUATION OF THE SURGICAL TREATMENT OF DIFFUSE OBSTRUCTIVE EMPHYSEMA[1, 2]

A Physiologic Study

BURTON S. TABAKIN, PRASANT K. ADHIKARI,[3] AND DONALD B. MILLER

(Received for publication April 29, 1959)

Introduction

The purpose of this paper is to evaluate on an objective basis the long-term results of surgical treatment in 18 patients with diffuse obstructive emphysema. This group was treated surgically between 1951 and 1956, and evaluation is based on pre- and postoperative lung function studies, including lung volumes, maximal breathing capacity, bronchospirometry, and analysis of arterial blood gases. The first postoperative studies were done four months to two years after surgery, and some patients were followed for a period as long as six years.

The results of surgery in patients with bullae or air cysts *without* obstructive disease, as reported in the literature, are excellent and will not be discussed in detail (1–6). Two such patients have been included in this paper for comparison only. An attempt will be made to show that surgical treatment of bullae in the presence of obstructive emphysema is feasible even in severely disabled patients and that the results of such surgery are encouraging. Many surgical techniques have been described in the literature (7–13). In the present group, surgery was aimed at the removal of air cysts with the least possible sacrifice of surrounding lung tissue. Section of the vagus nerve (14) was done, in addition, on 8 patients in the hope of diminishing bronchospasm.

Material

The material comprises 20 patients who have been grouped into three categories. *Group I* consists of 8 patients who, in addition to plication and removal of blebs, had vagus sections. *Group II*, comprising patients without vagus sections, is subdivided into *Group IIA* (unilateral surgery) and *Group IIB* (three bilateral procedures and one patient with two procedures on the same side). The 2 patients classified under *Group III* had surgery for localized blebs without obstructive disease and are included for comparison only. Eighteen of the 20 patients were males. The ages varied from thirty-six to sixty-one years, with an average of forty-two years. Clinically, all were seriously disabled, 12 being unable to work. Dyspnea was present in all, and some could not even walk on the level. Eleven patients were heavy smokers and had smoked more than one package of cigarettes a day for at least ten years. Only 6 had previous histories of bronchial asthma. Cough with expectoration was present in 18, 2 of whom had some bronchiectasis proved by bronchogram. Only one patient had signs of congestive cardiac failure.

Methods

The diagnosis of emphysema was established on the basis of the physical findings of increased postero-anterior diameter of the chest, diminished breath sounds, and hyper-resonance, in association with roentgenographic and fluoroscopic chest examination and pulmonary function studies as described below. The diagnosis of bullae or air cysts was made by roentgenographic examination and by function studies. A very high pulmonary mixing index was interpreted as suggestive evidence of bullae.

Maximal breathing capacity was determined by an open-circuit method using a high-velocity Rudolph valve and a 150-liter Douglas bag. Collections were made over a thirty-second period. Duplicate determinations were required to check within 5 liters. Volumes were calculated at ambient pressure and saturated with water vapor at body temperature (15).

The residual air and pulmonary volumes were determined by a modified open-circuit method of Darling, Cournand, and Richards (16). Duplicate studies were required to check within 5 per cent. Predicted values were calculated on the bases of age, height, and sex according to the method of Baldwin, Cournand, and Richards (17). Nitrogen analysis of the collected gas was done on the Van Slyke manometric apparatus, and alveolar samples were analyzed by the Van Slyke method and

[1] From the Cardiopulmonary Laboratory of the University of Vermont College of Medicine, the Mary Fletcher Hospital, and the departments of Medicine and Surgery of the University of Vermont, Burlington, Vermont.

[2] This study was supported in part by grants from the Vermont Heart Association and the Vermont Tuberculosis and Health Association.

[3] Fellow in Medicine, Cardiopulmonary Laboratory, University of Vermont College of Medicine, Burlington, Vermont.

TABLE 1

Complete Physiologic Data

Patient	Time	Clinical Status	Lung Volume in Liters				Nitrogen (after Oxygen)	Maximal Breathing Capacity	Rest or Exercise	Arterial Blood Studies							Alveolar Ventilation	Dead Space	Degree of Compression	Side
										Content Volume			Tension							
			Total Lung Volume	Vital Capacity	Mid-capacity	Residual Air Volume				Oxygen	Carbon Dioxide	Saturation	Oxygen	Carbon Dioxide	Alveolar Arterial Oxygen Difference	pH				
	(months)						(per cent)	(liters)		(per cent)			(mm. Hg)				(liters)	(cc.)		
Group I																				
1. (JK)	0	Dyspnea	6.66 (5.17)*	2.75 (3.91)	5.31 (1.95)	3.91 (1.26)	11.09	17 (136)	R	17.97	50.53	84.0	63	42	32	7.46	4.2	229	None	Left
	3	Well	6.95	4.21	4.13	2.74	4.71	54												No cysts
	21	Well	6.82	3.65	4.67	3.17	7.82	37	R	17.90	48.50	90.0	80	36	26	7.48	5.6	202		
2. (MT)	0	Dyspnea	5.52 (4.75)	2.83 (3.59)	3.54 (1.79)	2.69 (1.16)	6.74	38 (107)	E	16.21	58.44	84.0	59	44	36	7.48	11.3	290	None	Left
									R	15.63	59.65	82.0	66	46	18	7.46	3.6	200		Large bullae
	4	Well	4.89	3.00	2.81	1.89	3.8	37	E	17.71	53.01	88.0	69	46	22	7.41	6.6	349		
									R	16.82	53.70	91.0	71	43	24	7.42	2.8	150		
	18	Well	4.49	2.59	2.73	1.90	3.4	36												
3. (MG)	0	Dyspnea	4.45 (4.04)	2.09 (2.89)	3.25 (1.80)	2.36 (1.15)	4.45	16 (88)	R	18.38	55.14	88.0	73	40	28	7.50	2.6	85	1 plus	Right
		Well 1 year																		Many blebs
	19	Dyspnea recurred	4.90	1.49	4.15	3.41	5.76	12	R	16.31	56.13	92.0	80	40	30		4.1	132		
4. (RH)	0	Dyspnea	6.71 (4.79)	1.48 (3.62)	6.05 (1.80)	5.23 (1.17)	12.30	19 (106)											None	Right
	6	Well	7.24	1.95	6.39	5.29	19.99	21												Diffuse
	18	Died suddenly																		
5. (ES)	0	Dyspnea	6.18 (5.29)	2.54 (4.00)	4.74 (2.00)	3.64 (1.29)	9.9													Left
	7	Well	5.01	2.20	3.64	2.71	5.36	23 (103)												Multiple blebs
	16	Dyspneic																		
	36	Died																		
6. (MJ)	0	Dyspnea	7.83 (5.68)	2.33 (4.29)	6.66 (2.14)	5.47 (1.38)		28 (124)	R	16.80	59.50	88.0	65	51	11				None	Right
	6	Improved	6.92	2.25	5.25	4.35		36	R	17.33	56.80	94.0	82	48	25					Diffuse
	16	Dyspneic						28												
	24	Died in congestive failure																		
7. (JL)	− 24	Dyspnea	6.18 (5.66)	2.76 (4.28)	4.39 (2.14)	3.42 (1.38)	7.37	32 (154)												Right
	0		6.67	2.32	5.32	4.35	5.32	27	R	19.36	44.50	95.0	73	35	39		6.1	310	None	Diffuse
	9	Not improved	6.10	1.58	5.01	4.32	19.82		R	19.50	45.51	93.0	72	35	37		5.9	327		
8. (EP)	0	Dyspnea	7.04 (5.38)	2.01 (4.07)	5.85 (2.03)	5.03 (1.31)	16.46		R	15.93	60.87	88.0		50		7.42			Plus ±	Blebs
	4	Well	6.67	3.11	4.65	3.56	12.44		R				81	38	20		4.8	163		
									E	16.10	55.83	93.0	82	45	16	7.44	15.7	335	3 plus	Blebs
	12	Well	7.19	2.89	5.39	4.30	11.40	32 (144)												
2nd op.†	18	Dyspnea						28												
	24	Dyspnea	6.52	2.14	5.15	4.38	6.42	26	E	15.17	54.96	89.0	69	50	17	7.40	12.1	555		
	33	Died																		
1. (ER)	0	Dyspnea	7.28 (4.70)	3.11 (3.55)	5.17 (1.77)	4.17 (1.15)	15.87	30 (122)												
	3	Well	6.63	2.50	4.48	4.13	14.21	32											None	Right
	29	Well	6.25	1.87	4.99	4.38	18.95	23												Extensive Disease
2. (HA)	0	Dyspnea, cough	6.79 (5.60)	3.55 (4.23)	3.90 (2.11)	3.24 (1.37)	8.77	48 (137)												

	32	Died																				
Group IIA																						
1. (ER)	0	Dyspnea	7.28(4.70)	3.11(3.55)	5.17(1.77)	4.17(1.15)	15.87	30(122)												None	Right
	3	Well	6.63	2.50	4.48	4.13	14.21	32													Extensive
	20	Well	6.25	1.87	4.99	4.38	18.95	23													Disease
2. (HA)	0	Dyspnea, cough	6.79(5.60)	3.55(4.23)	3.90(2.11)	3.24(1.37)	8.77	48(137)												None	Right
	32	Slight dyspnea	5.86	2.95	3.41	2.91	0.82	42													Blebs
3. (TC)	0	Dyspnea	8.44(5.60)	4.43(4.23)	5.11(2.11)	4.01(1.37)	5.06	43(129)												3 plus	Left
	20	Improved	8.53	4.27	5.76	4.26	4.2	39													Bullae
4. (RL)	0	Dyspnea	7.48(6.15)	3.04(4.65)	5.78(2.32)	4.44(1.56)		34(128)	E	17.84	50.64	91.0								None	Right
									R	17.24	50.58	97.0									Blebs
	4	Marked improvement	6.83	3.30	4.95	3.53		28	E	17.88	49.01	90.0	62	48	23						
									R	17.46	49.24	97.5									
	48	Marked improvement	6.34	2.46	4.78	3.88	4.7	22													
5. (VB)	0	Dyspnea	6.43(4.68)	2.79(3.54)	4.64(1.77	3.64(1.14)	5.09	42(111)	E	16.66	45.18	84.0						34.1	395	2 plus	Left
	2							50	E	17.65	45.33	88.0		36(Alv)‡		7.45	31.6	428			Bullae
	12	Well	6.23	3.05	4.02	3.18	3.43	64													
6. (WB)	0	Dyspnea	4.46(5.45)	2.00(4.12)	3.08(2.06)	2.46(1.33)	13.00	22(116)												None	Right
	36	No dyspnea						23													Diffuse
	48	Cough—no dyspnea					12.00	24													No blebs
Group IIB																					
1. (RF)	0	Dyspnea	7.92(6.02)	2.05(4.55)	6.45(2.27)	5.87(1.47)	12.20	30(119)													Left
	10	Well	6.43	2.81	4.57	3.62	17.22	37												None	Cyst
	24	Well	5.16	2.49	3.58	2.67	11.68	28													
2nd op.	24	Dyspnea																			Left
	36	Well	5.00	1.66	3.83	3.34	18.01	19													Cyst
	57	Dyspnea	5.91	1.73	4.86	4.18	14.11	19													
2. (AS)	0	Pulmonary cripple																			
	35	Much improved	7.62(5.04)	3.04(3.81)	5.82(1.90)	4.58(1.23)	8.45	21(121)													
2nd op.	37	Dyspnea																		4 plus	
	66	Improved	7.64	2.25	6.42	5.39	8.40														
3. (RB)	0	Dyspnea	8.07(6.25)	3.31(4.72)	6.04(2.35)	4.76(1.52)		47(151)	E	18.61	49.48	96.8	77	34	27					2 plus	
									R				69	41	24						
	6	Well, but bronchorrhea	7.20	3.42	5.25	3.78	10.12	43	E	18.95	44.42	95.0	72	42	32	7.38	21.5	392		None	Left
									R	18.41	47.48	99.0	76	35	33	7.48	6.0	356			Bullae
2nd op.		Bronchorrhea																			
	24	Dyspnea	3.93	1.83	2.71	2.10	1.9	43	R	16.97	50.54	92.0								1 plus	Right
									E				60	35	51		18.8	210			Bullae
4. (RD)	0	Dyspnea	7.94(5.67)	4.27(4.29)	4.77(2.14)	3.67(1.38)	4.2	45(144)	E	17.31	40.76	90.0	68	37	36		42.0			Left 3 plus	Left
									R	15.92	43.84	92.5	70	38	31						Cyst
	3	Well	6.38	3.60	3.82	2.78	2.6	65													
2nd op.	3																				
	8	Well	5.16	3.49	2.40	1.67		104	E	17.78	41.48	96.0	99	40	32		36.0			Right 3 plus	Right
	17	Well																			Cyst
	30	Well						108													
	75	Well	5.68	3.26	3.00	2.32	1.6	110													
								102													
Group III																					
1. (GG)	0	Cough	7.79(6.67)	5.22(5.04)	3.91(2.52)	2.57(1.63)		136(167)	E	18.24	42.54	94.0	77	37	23					3 plus	Left
	16	Well	8.50	5.38	4.38	3.12		139	E	18.79	43.40	95.5	81	40	18						Cyst
	72	Well	7.29	4.85	3.91	2.44	2.2	118													
2. (ME)	0	No dyspnea	2.91(4.01)	1.83(2.87)	1.29(1.79)	1.08(1.14)	2.16	63(83)													
	35	No dyspnea	3.81	2.79	1.44	1.02	1.4	84													

* Figures in parentheses are predicted values † = Second operation ‡ = Alveolar trap sample

later in the study with a Waters A-6 nitrogen meter.

Exercise blood gas studies, when able to be performed, were done on a motor-driven treadmill, an indwelling Riley needle having previously been placed in the left brachial artery. Expired gas and arterial blood were collected simultaneously during the fifth and sixth minutes of exercise. Resting studies were done by collecting expired gas and arterial blood simultaneously after a period of adaptation to the mouthpiece. Expired gas was analyzed for oxygen and carbon dioxide on the Scholander apparatus. Duplicate analyses were required to check within 0.04 per cent. Minute volume was calculated at ambient pressure and saturated with water vapor at body temperature. Oxygen utilization and carbon dioxide production were calculated at standard temperature and pressure, dry.

Oxygen content and capacity of the arterial blood were determined on the Van Slyke apparatus by the method of Van Slyke and Neill (18), as was carbon dioxide content. Duplicate analyses were required to check within 0.25 volumes per cent. Direct tension of oxygen and carbon dioxide in the arterial blood was determined by the Filley, Gay, and Wright (19) modification of the Riley, Proemmel, and Franke method. Duplicate analyses checked within 3 mm. of mercury. Plasma carbon dioxide and pH were calculated from the whole blood carbon dioxide content, the hemoglobin capacity, and the carbon dioxide tension according to the method of Van Slyke and Sendroy (20). Alveolar oxygen tension was calculated by the modified Bohr formula of Filley, MacIntosh, and Wright (21).

Bronchospirometry was performed with a Carlens double-lumen catheter. Expired gas collections were made in two Douglas bags through a modified Collins bronchospirometer. Gas volume was measured and samples were analyzed in a Scholander apparatus. Ventilation, oxygen utilization, carbon dioxide production, and vital capacity were calculated for each lung as a percentage of the total. Multiple spirograms were obtained to establish adequate separation of the two lungs.

The operative procedures were tailored to the individual case and consisted of the removal of larger blebs and plication of the smaller ones. Great care was taken to avoid unnecessary loss of lung parenchyma. In individuals with bilateral bullae, the side showing the poorer function by bronchospirometry was operated on first. At the time of the surgery, the degree of compression of surrounding lung tissue was noted when possible and graded from one- to four-plus. Vagus section, when done, was based on a previously good response of the maximal breathing capacity to bronchodilator agent or the anesthetist's sensation of decreased resistance of the lung produced by xylocaine block of the vagus at the time of surgery.

Results

There were no operative deaths. All patients but one (*JL*) showed marked clinical improvement after the first procedure. Postoperative complications were hydropneumothorax in 2 patients and empyema in 2, one of each requiring thoracoplasty; one patient developed a transient psychosis in the early postoperative period. Four patients died between eighteen and thirty

TABLE 2

Results of Bronchospirometry Showing Function of the Surgically Treated Side Before and After Operation

Name	Time of Study	Operated Side	(Function in Per Cent of Whole)			
			Ventilation	Oxygen Uptake	Carbon Dioxide Output	Vital Capacity
	(*months*)					
Group I						
J.K.	0	Left	60	49	58	48
	21		52	53	52	49
M.T.	0	Left	29	28	26	39
	4		30	29	26	32
M.G.	0	Right	49	45	46	39
	23		52	58	56	52
J.L.	0	Right	41	42	39	69
	9		39	38	33	51
E.P.	0	Right	52	52	52	50
	4		55	62	56	49
	0	Left	46	46	43	49
	7		41	43	38	45
Group IIA						
R.L.	0	Right	43	45	44	52
	4		59	63	64	46
V.B.	0	Left	32	31	29	39
	2		50	51	52	45
	12		35	32	32	30
Group IIB						
R.F.	0	Left	52	34	40	39
	10		40	34	36	43
	0	Left	32	31	30	43
	12		29	34	23	33
R.B.	0	Left	40	50	49	43
	9		32	39	33	36
R.D.	0	Left	45	38	43	44
	3		53	54	57	40
	8	Right	48	46	46	47
Group III						
G.G.	0	Left	40	42	41	47
	16		46	47	47	42

TABLE 3

GAIN OR LOSS IN PER CENT OF FUNCTION ON THE SURGICALLY TREATED SIDE

Patient	Operated Side	Ventilation	Oxygen Uptake	Carbon Dioxide Output	Vital Capacity	Degree of Pulmonary Compression
Group I						
1. (J.K.)	Left	−8	+4	−6	+1	None
2. (M.T.)	Left	+1	+1	Same	−7	None
3. (M.G.)	Right	+3	+13	+10	+13	Plus
4. (J.L.)	Right	−2	−4	−6	+2	None
5. (E.P.)	Right	+3	+10	+4	−1	E.P. had two operations with vagus section on the left side only, R+; L+++.
	Left	−5	−3	−5	−4	
Group IIA						
6. (V.B.)	Left	+18	+20	+23	+6	1+
7. (R.L.)	Right	+16	+18	+20	−6	None
Group IIB						
8. (R.F.)	Left	−12	Same	−6	+4	R.F. had two operations on the same side—no compression.
	Left	−3	+3	−7	−10	None
9. (R.B.)	Left	−8	−11	−16	−7	R.B. had two operations but bronchospirometry not done before and after second surgery. L-none; R+.
10. (R.D.)	Left	+8	+17	+14	−4	R.D. had two operations. L+++
	Right	+5	+9	+11	−7	R+++
Group III						
11. (G.G.)	Left	+6	+5	+6	−5	3 +

months postoperatively. Causes of death were congestive failure in 2 patients (*MJ* and *ES*), sudden unexplained death in one (*RH*), and respiratory insufficiency in the fourth (*EP*) following a thoracoplasty for unexpanded lung.

Complete physiologic data are presented in tables 1, 2, and 3. In *Group I*, all but one patient were studied within nine months following surgery. The maximal breathing capacity increased in 3 and decreased in 2 patients, while 3 were unable to perform the test. Vital capacity and total lung volume increased in 4 patients and decreased in 4. Mid-capacity and residual air volumes decreased in 5 and increased in 3. Pulmonary mixing index decreased in 4 patients and increased in 3, and the data were not available in one.

Of 6 patients who had arterial blood studies, 5 showed a preoperative oxyhemoglobin saturation below 90 per cent either at rest or exercise. All were above 90 per cent in the postoperative evaluation. One patient included in *Group I* did fall to 89 per cent saturation following a second procedure. Arterial carbon dioxide tension determined at rest decreased in 4 patients in *Group I*, the largest decrease being 24 per cent.

The 6 patients of *Group IIA* were studied at intervals of six to thirty-six months postoperatively. Maximal breathing capacity increased in 3 patients and decreased in 3. Vital capacity increased in 2, decreased in 3, and could not be done in one. Total lung, mid-capacity, and residual air volumes decreased in 4 patients, increased in one, and could not be determined in one instance. Pulmonary mixing index decreased in 5, and the result is not available in one.

In one of the 2 patients in this group with arterial blood studies, the oxyhemoglobin saturation was less than 90 per cent both pre- and postoperatively. Carbon dioxide diminished in one patient following surgery.

Three patients in *Group IIB* were restudied within one year following each of their surgical procedures. Following the first operation, the maximal breathing capacity increased in 2 and was unchanged in one. Following the second operation, maximal breathing capacity increased in one, decreased in one, and was unchanged in one. Vital capacity after the first operation

increased in one patient, decreased in one, and was unchanged in one. Following the second procedure, all vital capacities decreased. Total lung, mid-capacity, and residual air volumes showed progressive decrease in all patients after the first operation. Mixing index increased in one, decreased in 2, and remained the same in one.

One of the 2 patients in *Group IIB* who had arterial blood studies showed minor depression in oxyhemoglobin saturation, and one showed a minor increase in arterial saturation after the second procedure. Carbon dioxide tension did not change significantly in either one. The results of bronchospirometry in 10 patients are shown in table 2, and the comparisons between the preoperative and postoperative studies are summarized in table 3. Ventilation increased in 8 instances and diminished in 5; oxygen utilization increased in 7, diminished in 2, and remained the same in one. Carbon dioxide production paralleled ventilation. Vital capacity increased in half and decreased in the other half. Second operations in patients *EP* and *RF* resulted in diminished function of the treated side.

Discussion

Surgical removal of emphysematous blebs might be expected to improve function by allowing the compressed lung to expand, by decreasing dead space and thereby increasing alveolar ventilation (22), and by relieving compression on the pulmonary vasculature (9). A combination of these effects might then be expected to return ventilation perfusion ratios toward normal. Relief of bronchospasm by nerve section should also improve alveolar ventilation. It should be pointed out, however, that objective evidence from lung function studies does not correlate closely with the clinical picture of pulmonary emphysema (23), and only minimal improvements in these studies may be observed postoperatively in patients with marked clinical improvement.

The patients in this report were treated surgically because of their clinical disability, objective evidence of functional impairment, relative youth, and failure of intensive medical therapy. Following surgery, 17 of the 18 patients had impressive relief of dyspnea, and many were able to return to productive activity.

The two objective factors common to this improvement were a reduction in residual air volume and pulmonary mixing index; this occurred in 13 and 11 instances, respectively. Vital capacity was raised in only 7 patients, and maximal breathing capacity increases were unimpressive, with only 2 exceptions. Surgical relief of pulmonary parenchymal compression showed no correlation with measurable changes in function. In fact, patients estimated at operation to have no compression exhibited some of the most definite postoperative clinical and functional improvement. Microscopic sections of resected lung tissue failed to reveal any evidence of alveolar wall thickening as a cause of arterial oxygen unsaturation.

In those patients demonstrating increased arterial oxyhemoglobin saturation after surgery, alveolar ventilation was only occasionally increased, and physiologic dead space changes varied. These facts would strongly suggest that more normal ventilation-perfusion balance has been approached. Bronchospirometric studies showing almost universal increases in oxygen uptake on the operated side despite erratic changes in ventilation add further weight to this hypothesis. That those patients with the greatest clinical improvement had undergone vagus section would also indicate that this procedure had definite value.

Of the 8 patients studied a second time after surgery, 6 showed definite deterioration in function when the data were compared with the results of the first postoperative study, but the patients were still improved over the preoperative level. These patients all maintained their good clinical improvement. The deterioration in function would suggest progression of the original disease process. There appeared to be no impairment of function from the surgical procedure itself.

From this presentation, certain conclusions may be drawn. Although no definite criteria for selection of patients for surgery can be laid down, it is believed that severely disabled patients of relatively younger age with some degree of bronchospasm and at least suggestive evidence of bullae by roentgenographic examination or studies of pulmonary function should be offered surgical therapy. At the time of operation, effort should be directed to removing or plicating larger blebs. Vagus section should be done if there is an element of bronchospasm as shown by improvement of maximal breathing capacity after bronchodilator agents. The results in the

present group show that this surgical procedure offers definite benefit to patients with diffuse obstructive emphysema complicated by bullae.

Summary

Preoperative and long-term follow-up function studies are presented of 18 patients with diffuse obstructive pulmonary emphysema with bullae. All of the patients but one showed good clinical improvement, although 4 died eighteen to thirty-six months later from ultimate progression of their disease. The major pulmonary function changes were definite decreases in residual air and pulmonary mixing indices and increases in arterial oxyhemoglobin saturation. Improvement was associated with increased oxygen uptake on the affected side as demonstrated by bronchospirometry. Vagus section was associated with clinical improvement.

Improvement in ventilation perfusion ratio is suggested as the basis of the demonstrated clinical improvement.

Sumaeio

Justipreciación Objetiva a Largo Plazo del Tratamiento Quirúrgico del Enfisema Oclusivo Difuso, Estudio Fisiológico

Preséntanse estudios funcionales preoperatorios y de observación a largo plazo en 18 enfermos que tenían enfisema pulmonar oclusivo difuso con flictenas. Todos los enfermos, menos uno, revelaron buena mejoría clínica, aunque 4 fallecieron de dieciocho a treinta y seis meses después de agravación final de su enfermedad. Las mayores alteraciones de la función pulmonar consistieron en disminuciones bien definidas de los índices del aire residual y de la mezcla pulmonar y aumentos en la saturación arterial de la hemoglobina. La mejoría se vinculó con aumento de la toma de oxígeno en el lado afectado según reveló la broncospirometría. La resección del neumogástrico se asoció con mejoría clínica. Como base de la mejoría clínica observada se sugiere el mejoramiento de la proporción de ventilación y perfusión.

Resume

Evaluation objective à long terme du traitement chirurgical de l'emphysème oblitérant diffus. Une étude physiologique

Les auteurs présentent une étude du fonctionnement pulmonaire à la période pré-opératoire et pendant une longue durée après l'intervention chez 18 patients atteints d'emphysème pulmonaire bulleux, oblitérant diffus. Tous les malades sauf un ont présenté une amélioration clinique nette, bien que 4 d'entre eux soient décédés de 18 à 36 mois après l'intervention, par suite de l'aggravation finale de leur maladie. Les changements les plus importants du fonctionnement pulmonaire étaient: une diminution nette de l'air résiduel ainsi qu'une augmentation de la saturation de l'oxyhémoglobine artérielle. L'amélioration fut imputée à un apport en oxygène accru du côté atteint, comme le montrait la bronchospirométrie. La pneumogastrectomie a été suivie d'une amélioration clinique. Il est suggéré que cette amélioration clinique est due à une amélioration du pourcentage de la perfusion de l'oxygène.

Acknowledgments

The writers wish to express their appreciation to Drs. George W. Wright and Giles F. Filley for performing at Trudeau, and allowing the use of, the initial study on patient *R.B.*, the first two studies on *M.J.*, *R.L.*, and *G.G.*, and the first three studies on *R.D.* The writers are most grateful to Dr. Wright for his critical and extremely helpful comments during the preparation of the manuscript. The technical assistance of Leona Bessee, R.N., and Reba J. Beecher, R.N., was invaluable.

REFERENCES

(1) Baldwin, E. D., Harden, K. A., Greene, D. G., Cournand, A. S., and Richards, D. W., Jr.: Pulmonary insufficiency: IV. A study of sixteen cases of large air cysts or bullae, Medicine, 1950, *29*, 169.

(2) Oughterson, A. W., and Taffel, M.: Pulmonary cysts: Review of subject with a case report, Yale J. Biol. & Med., 1936–1937, *9*, 77.

(3) Albritten, F. F., Jr., and Templeton, J. Y.: Treatment of giant cyst of the lung, J. Thoracic Surg., 1950, *20*, 749.

(4) Cooke, F. N., and Schaff, B.: The surgical management of emphysematous blebs and bullae, South. M. J., 1953, *46*, 474.

(5) Jewsbury, P.: Surgical treatment of cystic disease and emphysema in young children, Brit. J. Surg., 1955, *42*, 601.

(6) Weisel, W.: Surgical treatment of localized emphysematous disease, Ann. Surg., 1955, *142*, 17.

(7) Stringer, C. J., and Burnet, C. A.: Surgical treatment of emphysematous bullae, Am. Rev. Tuberc., 1956, *74*, 856.

(8) Warring, F. C., Jr., and Lindskog, G. E.: Surgical management of giant air cysts of the lung, Am. Rev. Tuberc., 1951, *63*, 579.

(9) FitzPatrick, M. J., Kittle, C. F., Lin, T. K., and Dowell, J. C.: Some physiologic changes associated with surgical excision of emphysematous bullae, Am. J. Med., 1957, *22*, 534.

(10) Dugan, D. J., and Samson, P. C.: Surgical treatment of giant emphysematous blebs and pulmonary tension cyst, J. Thoracic Surg., 1950, *20*, 729.

(11) Clagett, O. T.: Surgical treatment of emphysematous blebs and bullae, Dis. Chest, 1949, *15*, 669.

(12) Naclerio, E., and Langer, L.: Pulmonary cysts: Special reference to surgical treatment of emphysematous blebs or bullae, Surgery, 1947, *22*, 516.

(13) Walkup, H. E., and Wolcott, M. W.: Surgical treatment of bullous emphysema, Dis. Chest, 1955, *28*, 638.

(14) Abbot, O. A., Hopkins, W. A., and Guilfoil, P. H.: Therapeutic status of pulmonary autonomic nerve surgery, J. Thoracic Surg., 1950, *20*, 571.

(15) Methods in Medical Research, Vol. II, J. H. Comroe, Jr., ed., Year Book Publishers, Chicago, 1959, p. 213.

(16) Darling, R. C., Cournand, A., and Richards, D. W., Jr.: Studies on intrapulmonary mixture of gases: III. Open circuit method for measuring residual air, J. Clin. Invest., 1940, *19*, 609.

(17) Baldwin, E. D., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency, Medicine, 1948, *27*, 243.

(18) Van Slyke, D. D., and Neill, J. M.: Determination of gases in blood and other solutions by vacuum extraction and manometric measurement, J. Biol. Chem., 1924, *61*, 523.

(19) Filley, G. F., Gay, E., and Wright, G. W.: Accuracies of direct determination of oxygen and carbon dioxide tension in human blood *in vitro*, J. Clin. Invest., 1954, *33*, 510.

(20) Van Slyke, D. D., and Sendroy, J., Jr.: Line charts for graphic calculation by Henderson Hasselbalk equation and for calculating plasma carbon dioxide content from whole blood content, J. Biol. Chem., 1928, *79*, 781.

(21) Filley, G. F., MacIntosh, D. J., and Wright, G. W.: Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise, J. Clin. Invest., 1954, *33*, 530.

(22) Dowell, J. C., and FitzPatrick, M. J.: Cardiopulmonary function in bullous emphysema, Dis. Chest., 1957, *32*, 162.

(23) Baldwin, E. D., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: III, Medicine, 1949, *28*, 201.

PULMONARY INVOLVEMENT IN LEUKEMIA[1]

ROBERT A. GREEN[2] AND NORMAN J. NICHOLS

(Received for publication April 20, 1959)

INTRODUCTION

The writers recently studied and reported (1) a case of chronic lymphatic leukemia in which massive infiltration of the lung by leukemic cells caused fatal pulmonary insufficiency of the alveolar-capillary block type. Prompted by this case and by the discussion it stimulated, the previous experience with pulmonary involvement in leukemia at this hospital was reviewed in an attempt to determine its incidence and to correlate the pathologic with the clinical and roentgenographic findings.

PLAN OF STUDY

The autopsy protocols, roentgenographic reports, and summaries of clinical records of all patients upon whom autopsies were performed between mid-1946 and mid-1957 and in whom a diagnosis of leukemia was made were reviewed. If gross or histologic pulmonary abnormalities were described in the autopsy protocol, or if unusual clinical or roentgenographic pulmonary findings were mentioned in the summaries, a more complete study of the case was made. This consisted of evaluation of the clinical records with specific attention to symptoms and findings in the lungs; of study of the chest roentgenograms, when still available; and of critical review of the microscopic sections. If, following the review, the diagnosis in certain patients seemed more compatible with lymphomas other than leukemia, or with myeloproliferative disorders which terminated in a leukemic phase, the cases were discarded. A total of 109 patients remained for inclusion in this report.

Leukemia was then subdivided into the acute and chronic forms; acute exacerbation or blastic crisis of chronic leukemia was included with the latter (*see* table 1). In the majority of cases subdivision was easily made by clinical, hematologic, pathologic, and temporal criteria. In other words, it was found that patients with bone marrow consisting predominantly of large numbers of one type of immature leukocyte and showing minor degrees of organ infiltration usually had run an acute course, death occurring within six to nine months of onset of clinical disease. On the other hand, patients with marrow showing a more variegated picture, as well as considerable visceral involvement, ordinarily had manifested a more chronic illness lasting well over a year. In a few instances, however, patients with hematologic and pathologic findings suggesting chronic disease ran a rather fulminant course; the reverse situation was quite rare. These few cases, many of which had previously been called "subacute," were classified as either chronic or acute leukemia depending upon the entire picture, greater emphasis being placed upon hematologic findings as opposed to the duration of the disease when it was necessary to discriminate between the two.

Further subdivision into myelogenous leukemia and lymphatic leukemia was made predominantly upon the basis of the description of the hematologic findings, especially of the bone marrow aspirate taken during life, although the vast majority of the marrow slides were no longer available for review. Other subdivisions, such as acute stem cell and acute monocytic leukemia, were therefore of necessity listed in table 1. Although the specific sub-diagnosis in these few patients may be open to some question, it is believed that the over-all classification of the patients in this series is quite reliable.

As only autopsy cases were included in the study, interpretation of chest roentgenograms was facilitated by having the gross descriptions of the lungs as well as the microscopic sections available for complete correlation. Evaluation of the clinical records in terms of the cause of various pulmonary symptoms, however, was more difficult. Critical review indicated that dyspnea, the symptom in which there was the most interest, was usually related to the anemia so often present in leukemic patients, rather than to specific pulmonary pathology. Similarly, various degrees of pulmonary infection, from mild bronchitis to overwhelming pneumonia, occurred with great frequency in these patients, and was commonly manifested by such symptoms as fever, cough, sputum, and variable degrees of dyspnea. Likewise, thrombocytopenia usually accounted for pulmonary hemorrhage, from mild degrees of streaking of the sputum to profuse hemoptysis.

The greatest difficulties encountered in the study were in the interpretation and evaluation of the histologic preparations. An attempt was made to establish fairly rigid criteria for the diagnosis of "leukemic infiltrate." Demonstration was required that cells morphologically compatible with leukemic cells were located in extra-

[1] From the Chest Section of the Medical Service and the Cardiopulmonary Laboratory, Veterans Administration Hospital, Bronx, New York, New York.

[2] Present address: Veterans Administration Hospital, Ann Arbor, Michigan.

vascular collections which were distinctly unrelated to areas of apparent response to infection. The requirement that the cells be extravascular was made because the writers believed that intense congestion of the alveolar capillaries themselves with leukemic cells should not be considered as true infiltration of the lung, as the cellular detail within the lumina of the body capillaries reflects in large part the status of the peripheral blood, whereas "infiltration" seems to indicate involvement of the parenchyma of an organ.

Unfortunately, as far as infection is concerned, the cellular response in the lung in leukemia is often quite atypical, apparently partially due to the inability of the leukemic marrow to respond normally with an outpouring of polymorphonuclear leukocytes. This was found to be true in this series in all varieties of leukemia, and was especially frequent in instances of chronic myelogenous leukemia. Therefore, when lesions were located around small bronchi, and fibrinous material, necrotic and granular debris, bacterial colonies, and the like were present in the alveolar spaces, a diagnosis of bronchopneumonia was made when the cellular constituents of the lesions per se might have suggested infiltration with leukemic cells rather than response to bacterial infection.

Pulmonary hemorrhage was noted occasionally in this series, associated with hemorrhages in other viscera and connective tissues. The hemorrhagic areas in the lung contained leukocytes apparently proportionate to the number of leukocytes seen histologically within the larger pulmonary vessels in the sections. Occasionally, a sedimentation or settling-out of the leukocytes was noted, and this mimicked leukemic infiltration when it occurred within a hemorrhagic area. Lesions of this type were not considered to be leukemic infiltrates.

When the larger pulmonary vessels were noted to be quite full of leukocytes in relation to erythrocytes, the pulmonary alveolar capillaries were often also quite cellular. As the alveolar-capillary membrane is ordinarily quite thin, a major difficulty existed in the determination of whether or not the cells seen were intraluminal or extraluminal in location as, by the criteria of the study mentioned above, the former were not considered to be leukemic infiltrates. The difficulty was compounded by the very frequent compression of alveolar septa found in histologic preparations. This has a multiplicity of causes, including artefacts associated with the opening of the thorax at autopsy; handling of the lung; and preparation of the sections, as well as *in vivo* factors including compression of areas of lung by pleural disease or an elevated diaphragm; pulmonary congestion; antecedent pulmonary disease, such as localized areas of pulmonary fibrosis or contraction of parenchyma due to organization of previous inflammation; and possibly unequal pulmonary ventilation in different areas during life due to obstruction of bronchioles by secretions. In areas of chronic pulmonary inflammation, alveolar lining cells often enlarge and take on a cuboidal shape; occasionally, these cells then resemble adjacent myeloid cells. In any case, an attempt was made to be quite strict in the diagnosis of leukemic infiltration if compression or any other pathologic change of these types was found.

Observations

Findings

A total of 109 patients with leukemia were studied, 48 of whom had acute leukemia and 61 some form of chronic leukemia (table 1). All of the patients with acute leukemia and all but 3 with chronic leukemia were males. The patients' ages ranged from the third to the eighth decade; 65 per cent of the patients with acute leukemia were less than forty years of age, whereas 77 per cent of the patients with chronic leukemia were more than forty; and most, in fact, were more than fifty years of age. Subdivision into types of acute and chronic leukemia is also given in table 1. The preponderant form of acute leukemia was myelogenous, with a few other cases listed as lymphatic, stem cell, or monocytic. As discussed previously, the criteria for the latter two subdivisions could not be critically reviewed. The chronic leukemias were more evenly divided between myelogenous and lymphatic forms, with a slightly greater incidence of the former.

TABLE 1

Leukemic Pulmonary Involvement

Classification and Number of Cases	Total Involvement		Involvement of Clinical Significance	
	Number	Per Cent	Number	Per Cent
Acute				
Myelogenous (34)	9	26	1	3
Lymphatic (9)	3	33	1	11
Stem (3)	1	33	—	—
Monocytic (2)	2	100	—	—
Total acute (48)	15	31	2	4
Chronic				
Myelogenous (29)	4	14	1	3
Lymphatic (23)	9	39	4	17
Acute exacerbation				
Myelogenous (8)	2	25	1	12
Lymphatic (1)	—	—	—	—
Total chronic (61)	15	25	6	10
Total 109	30	27	8	7

Pulmonary Involvement

It may be seen in table 1 that the incidence of leukemic involvement of the lung of all types in this study was 27 per cent. There was no significant difference in incidence of involvement between the patients with acute leukemia, all types (31 per cent), and those with chronic leukemia, all types (25 per cent). Similarly, no true difference was noted among the small number of patients in the various subgroups of acute leukemia. In the patients with chronic leukemia, however, the incidence of pulmonary infiltration was significantly greater in those with the lymphatic form.

It is also shown in table 1 that, although the total incidence of leukemic pulmonary infiltration in this series was 27 per cent, the incidence of leukemic involvement of *clinical significance*, as manifested by the presence of symptoms, signs, roentgenographic findings, and difficulties in differential diagnosis, was much lower, only 7 per cent of the entire series. Although the number of cases is small, it appears that clinically significant involvement occurs with greater frequency in chronic leukemia (10 per cent) as compared with the acute form (4 per cent). The difference between chronic myelogenous and chronic lymphatic leukemia may be even more striking. In the 9 patients with chronic lymphatic leukemia and pulmonary infiltration, the involvement was of clinical significance in 4; and in all patients with chronic lymphatic leukemia the incidence of clinically important leukemic infiltration was 17 per cent.

The schema in table 2 was devised and found to be practical in classifying the various types of leukemic infiltration of the lungs. The major anatomic division is into "parenchymal" and "nonparenchmyal" infiltration. "Nonparenchymal" infiltration refers to involvement primarily of the supporting and connecting structures of the lung, such as the bronchi, the blood vessels, the pleura, and their surrounding tissues. The term "parenchymal" infiltration is used to refer to lesions which appear to involve predominantly the alveolar septa and alveolar spaces without regard to broachovascular structures. In the discussion which follows, the symbols in parenthesis refer to the letters and numbers of the classification in table 2.

"Parenchymal" involvement: Diffuse interstitial parenchymal infiltration of considerable degree

TABLE 2

CLASSIFICATION OF LEUKEMIC PULMONARY INVOLVEMENT AND INCIDENCE OF VARIOUS TYPES

	Number of Patients	Incidence (per cent)
A. Parenchymal		
1. Diffuse interstitial		
a. Major	1	0.9
b. Minor	3	2.7
2. Nodular		
a. Major (gross)	1	0.9
b. Minor (microscopic)	2*	1.8
B. Nonparenchymal		
1. Bronchial and peribronchial		
a. massive	—	—
b. focal	4	3.7
2. Perivascular		
a. massive	—	—
b. focal	2	1.8
3. Pleural and subpleural		
a. massive	4	3.7
b. focal	4	3.7
4. Diffuse (combinations of types 1, 2, 3)		
a. massive	2	1.8
b. focal	6	5.5
C. Pulmonary infarction	3†	2.7
Totals	30*†	27

* One of these patients had massive pleural disease.

† One patient also had pleural infiltration.

(table 2, A1*a*) was found in one case in this series. This is the patient with the syndrome of alveolar capillary block described in another report (1).

Three instances were found in which diffuse interstitial parenchymal infiltration was present, but in which the infiltration was considered to be of minor extent (table 2, A1*b*). In all 3 cases the infiltration at first appeared to be extensive; however, further study revealed that all of the pulmonary blood vessels in these patients, including the alveolar capillaries, were literally choked with leukemic cells. Under high magnification erythrocytes were interspersed with the early leukocytes in all areas, so that most of the cellularity of the lung picture was intravascular. However, in many areas individual cells were distinctly noted to be outside the lumina of the capillaries as well as in alveolar spaces; for this

reason the involvement was classified as diffuse interstitial of minor degree (figure 1).

One of the 3 patients in this group had chronic myelogenous leukemia, and the other 2 are the 2 cases listed as acute monocytic leukemia. Neither patient with acute leukemia had symptoms or roentgenographic findings; however, the patient with chronic myelogenous leukemia had roentgenographic evidence of diffuse bilateral reticular pulmonary infiltration without specific symptomatology one month prior to death. These roentgenographic findings regressed considerably thereafter on no specific therapy. It is thus conceivable that transient leukemic infiltration resulted in the earlier roentgenographic appearance, and that the residuals were noted in the histologic material.

Scattered true nodules of parenchymal leukemic infiltration were found in 3 patients (table 2, A2), none of whom had pulmonary symptoms. In one, the nodules in the lungs were seen grossly at the time of autopsy and were

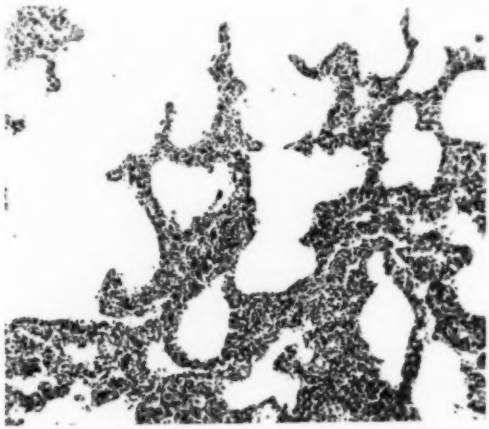

FIG. 1. Diffuse interstitial infiltration, minor degree. Note vessel in center of field. (×98)

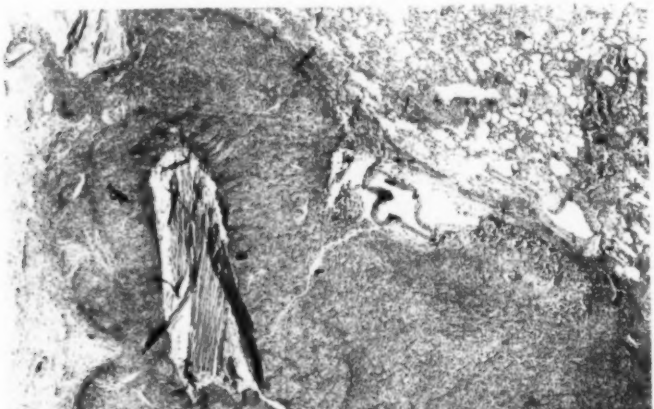

FIG. 2. Nodular infiltrate, macroscopic. (×15)

described as multiple, firm, white foci, 2.0 to 3.0 cm. in size. Unfortunately, the roentgenogram of this patient was not available for review. Microscopically, the lesions consisted of densely packed lymphocytic cells which in areas were diffusely infiltrating the surrounding lung, but elsewhere were more sharply demarcated from the lung and were compressing it (figure 2). No alveolar structures were seen within the lesions, but remnants of degenerating bronchial cartilage and vascular and lymphatic spaces were identified (figure 2). It is thus possible that the lesions arose from the peribronchial connective tissues and extended into the lung from these areas.

In the other 2 patients, the nodules were noted only microscopically. In a patient with chronic myelogenous leukemia they consisted of small, quite well-circumscribed groups of myeloid cells, with some local collection of erythrocytes, located within both alveolar walls and spaces. The last patient had chronic lymphatic leukemia with massive pleural involvement, and small microscopic collections of lymphocytes were found scattered throughout the lungs. The lungs were normal in the chest roentgenograms of both patients.

"Nonparenchymal" involvement: Involvement limited to bronchi and peribronchial tissues occurred in 4 patients (table 2, B1). Although usually the cells were located external to the cartilage in the peribronchial tissues, occasionally the infiltration involved the entire thickness of the bronchial wall, including the mucosa and submucosa. Both large and small bronchi were involved, the latter more frequently. In all 4 patients, only a few scattered bronchi were involved, and in no patient was the total extent of infiltration considerable. None of the patients had symptoms or findings referable to the leukemic infiltration.

In 2 patients, perivascular connective tissues were exclusively involved (table 2, B2), but in neither instance was the infiltration generalized or of major degree, nor were related clinical or roentgenographic findings present.

The commonest area of lung in which leukemic infiltration occurred in this series was the pleura and subpleural tissue. A total of 16 patients, an incidence of 15 per cent, was found, and in 6 of these the involvement was of considerable extent. In half of the total group the leukemia in the lung was limited to the pleura (table 2, B3), and in the other half pleural infiltration was associated with other areas of infiltration in the perivascular and peribronchial tissues (table 2, B4). In 2 patients the degree of diffuse nonparenchymal involvement was of considerable extent (table 2, B4*a*), but neither had related clinical or roentgenographic findings. The same was true of 6 patients in whom the diffuse nonparenchymal infiltration was of minor degree (table 2, B4*b*), and occurred in scattered foci, and of 3 of the 4 patients in whom isolated pleural involvement of minor degree (table 2, B3*b*) was found. The fourth patient had recurrent bloody pleural effusion associated with his chronic myelogenous leukemia, and at autopsy areas of pulmonary infarction were found that were probably etiologically more important than the few subpleural focal collections of leukemic cells that were also noted.

In 4 patients the leukemic involvement was predominantly localized to the pleura and was of major degree (table 2, B3*a*). A patient with an acute exacerbation of chronic myelogenous leukemia had a moderately large pleural roentgenographic shadow that did not change in size for the three months prior to his death. At autopsy a pleural effusion and a greatly thickened pleura were found, which microscopically showed marked fibrosis and, underlying this zone, diffuse infiltration with leukemic cells. In areas the subjacent lung was also infiltrated. It is possible in this patient, as in others of this type, that a specific or nonspecific pleural inflammation had resulted in scarring and that the leukemic cells later involved the area, rather than being responsible for the pleurisy; however, there is no evidence for this viewpoint. The other 3 patients with major involvement limited to the pleura all had chronic lymphatic leukemia. In 2 the clinical manifestation of the leukemic involvement was the presence of progressive unilateral pleural effusion, bloody in one instance. The histologic picture was variable, areas of infiltration of leukemic cells alternating with acellular fibrosis in one patient, and multiple areas of massive nodular infiltration in the other. The third patient presented clinically with massive pleural disease and effusion, and the pleural manifestations dominated the entire clinical picture (figure 3). At autopsy the pleura was tremendously thickened by leukemic infiltration (figure 4), which microscopically (figure 5)

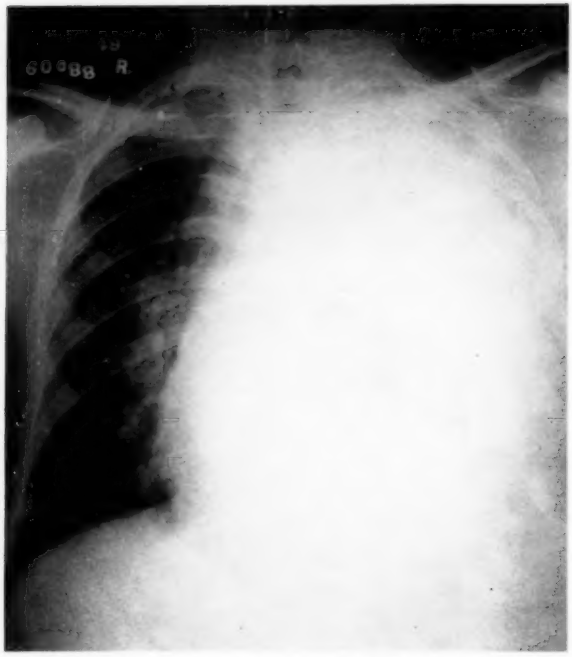

FIG. 3. Chest roentgenogram, postero-anterior view. Left pleural effusion, hilar and mediastinal adenopathy.

extended into all of the interlobular septa. This patient also had scattered foci of nodular parenchymal infiltration.

Pulmonary Infarction

One other type of pulmonary disease, namely pulmonary infarction (table 2, C), unrelated to the presence of cardiac disease or known peripheral thrombotic phenomena, was found on three occasions in this series. One patient with chronic myelogenous leukemia, mentioned above, had multiple small areas of infarction associated with a sanguineous pleural effusion; one patient with acute myelogenous leukemia developed a basilar infiltrate shortly before death which was diagnosed as bronchopneumonia but which proved to be an infarction surrounded by infiltration with leukemic cells at autopsy; and one patient with acute myelogenous leukemia had a large area of infarction with no apparent clinical manifestations.

Clinical Analysis

It is of considerable importance to the clinician to realize that the manifestations of leukemia in the lung and pleura can be of clinical significance in a certain percentage of cases. In tables 3 and 4 the entire group is presented more from the clinical than from the pathologic standpoint. Pulmonary infiltration which appeared during the course of the illness was analyzed to see how often leukemia was responsible for the findings, and the results are charted in table 3; the instances of pleural disease and effusion are treated similarly in table 4. The infiltrates in these patients were usually diffuse and bilateral, although occasional instances of more localized disease occurred in one lung. Ordinarily, the pulmonary findings developed toward the end of the course of the leukemia and, in all cases, were present at the time of death. The pleural effusions, on the other hand, were more often unilateral than bilateral, and more often the effusions

FIG. 4. Hemisection of left lung at autopsy. Note massive pleural thickening.

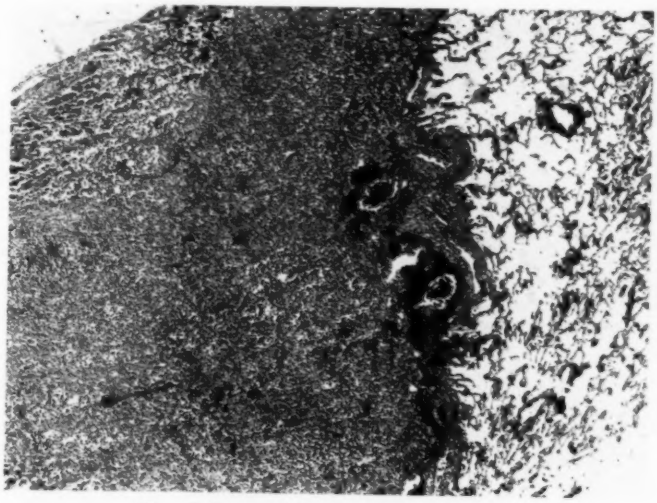

FIG. 5. Dense pleural infiltration. (×27)

TABLE 3

ETIOLOGY OF ROENTGENOGRAPHIC PULMONARY INFILTRATION

(21 Patients)

Leukemia		2
Infection		10
Bronchopneumonia	4	
Abscesses	3	
Tuberculosis	1	
Fungal infection	2	
Other		9
Hemorrhage	1	
Congestion and edema	3	
Infarction	1	
Fibrosis	2	
Idiopathic interstitial	1	
Silicotic nodules	1	
Normal	2	
Roentgenographic technique	1	
Pleural disease	1	
		21

TABLE 4

ETIOLOGY OF CLINICALLY DIAGNOSED PLEURAL DISEASE AND EFFUSION

(11 Patients)

Leukemia		5
Infiltration	4	
Massive intrathoracic lymphadenopathy without pleural infiltration	1	
Infections		4
Bronchopneumonia	1	
Tuberculosis	3	
Other		2
Infarction	1	
Heart failure	1	
		11

developed earlier than in the pre-terminal phase, although they were still present at the time of autopsy. The major criterion for the inclusion of a patient in these tables, in the case of both the pleural disease and parenchymal disease groups, was the fact that the clinical record indicated that leukemic infiltration had been seriously considered by the clinical staff or the radiologists in the differential diagnosis of the findings during life.

Pulmonary infiltration of this type was noted in 21 patients, 12 of whom had acute leukemia, and 9, chronic leukemia. In only 2 instances were the findings due to actual leukemic involvement. Most often infection was responsible (48 per cent); this was of various types, usually due to pyogenic organisms. Congestion and edema due to heart failure were also relatively frequent. A number of other pathologic findings, such as diffuse pulmonary hemorrhage, occurred on occasion, as noted in table 3.

Eleven patients developed pleural disease with effusion that presented a diagnostic problem. Only one patient in this group had acute leukemia. Unlike the parenchymal disease group, in which leukemia was uncommon as a cause of the findings, leukemic involvement of the pleura was the cause of the effusion in almost half the cases. Infections, especially tuberculosis, were also frequent, as noted in table 4.

DISCUSSION

An analysis of the data demonstrates that leukemic involvement of the lungs is not uncommonly found at necropsy, occurring in 27 per cent of the patients in the present series. This is at variance with the opinion generally held by most clinicians and radiologists (2, 3), although it was possible to find only a few previous studies of this subject in the literature. Vieta and Craver (4) reported 31 autopsied cases of lymphatic leukemia, of whom 19 per cent had parenchymal pulmonary involvement, whereas, of 23 autopsied cases of myelogenous leukemia, the parenchyma was involved in 13 per cent. Falconer and Leonard (5), in a study limited to lymphatic leukemia, reported an incidence of parenchymal involvement of 30 per cent. Nathan and Sanders (6) reviewed 59 autopsied cases of all types of acute leukemia and noted an incidence of 23 per cent.

In these studies the criteria for diagnosis of leukemic pulmonary involvement are either not discussed, or differ from criteria in the present report; however, although the number of cases in each is small, it is of interest that the incidence of leukemic pulmonary involvement is approximately the same. However, in a recently reported study, Cattaneo and Vercillo (7) noted an incidence of pleural or bronchopulmonary involvement in leukemia of 4 per cent (11 of 275 cases). Other etiologies of pulmonary abnormality, such as pneumonia, hemorrhage, and congestion, were carefully excluded from their tables. Of the eleven patients, four had acute leukemia, six chronic lymphatic leukemia, and one chronic myelogenous leukemia. They believe that the apparent approximate incidence of 25

per cent reported in the other studies referred to above is probably not accurate because of the small number of cases in each series, the lack of uniform criteria among various writers, and the small incidence from their own carefully studied group. Yet complete autopsy reports were available only in a small percentage of their cases, and ten of their eleven patients with leukemic involvement were discovered by roentgenographic examination. It may be that more complete histologic study would have revealed an incidence higher than 4 per cent; their figure more closely approximates that in the present study—7 per cent of infiltrates of *clinical significance*. A figure of 25 per cent may, therefore, approximate the true incidence of leukemic infiltration of the lung.

In the present study, no significant difference in incidence of pulmonary involvement between chronic and acute leukemia was found (*see* table 1). Likewise, no significant difference among the various subgroups of acute leukemia was noted. Although the 2 patients diagnosed as having acute monocytic leukemia both had diffuse interstitial involvement, albeit of minor degree, it is believed that no conclusion can be drawn regarding this apparent association not only because of the small number of cases, but also because the criteria for differentiation of acute monocytic from acute myelomonocytic and from acute myelogenous leukemia differed among the various hematologists who studied these patients over the years. It is of interest in this regard, however, that the only type of leukemia described by Liebow (8) as involving the lung is the acute monocytic form. The other studies referred to above note no difference in incidence among the subgroups of acute leukemia.

In contrast to this similarity, a distinct difference was apparent in chronic leukemia. The incidence was 39 per cent in chronic lymphatic leukemia and 14 per cent in chronic myelogenous leukemia, which is a slightly greater difference in the same direction than Vieta and Craver (4) found in their autopsy series (19 and 13 per cent, respectively).

The various sites of pulmonary infiltration—peribronchial, perivascular, pleural, and interstitial—are well known to pathologists. The pleural and subpleural localization was most common in this series. This is in contrast to Nathan and Sanders' report (6), in which infiltration of the alveolar septa was most common. The difference may be ascribed to the rigid exclusion by the present writers of cases in which compression of lung simulated infiltration and of those in which the "infiltration" of leukemic cells was only intravascular. It may be that this actually does represent a form of leukemic "infiltration" but, in the cases in which this type of apparent "involvement" was extreme, neither the roentgenographic nor clinical evidence suggested that any major pulmonary disease existed.

Previous studies of the lung in leukemia did not attempt to correlate the clinical, roentgenographic, and pathologic data. Kirklin and Hefke (9) reported a series of leukemic patients, four of whom had definite roentgenographic evidence of pulmonary infiltration. In one case, the infiltration resembled an abscess and disappeared following irradiation. This was considered proof of the leukemic nature of the pulmonary involvement. In the other 3 cases, no statement is made of whether pathologic examination was later performed, or if substantiation of the roentgenographic diagnosis was obtained. Vieta and Craver (4) reported a large group of patients with leukemia in whom roentgenographic abnormalities occurred. Twenty-seven per cent of the patients with lymphatic leukemia demonstrated pulmonary infiltration, whereas infiltrates were present in 7.5 per cent of the patients with myelogenous leukemia. These findings, however, were not correlated with the autopsy material presented in the same paper.

It is striking in this connection that the roentgenographic incidence of pulmonary infiltrations (27 per cent) in lymphatic leukemia is higher than the autopsy incidence (19 per cent); whereas with myelogenous leukemia, the reverse is true, only 7.5 per cent showing roentgenographic abnormalities, while 13 per cent were involved at autopsy, thus illustrating the need for correlation in each case. In only one of the nine patients described by Falconer and Leonard (5) as having pulmonary infiltrations at autopsy did the roentgenogram indicate an abnormality; and Nathan and Sanders (6) noted no roentgenographic involvement which could be attributed to leukemia in their 59 autopsied cases, 14 of which were cases with infiltration. They commented that this might be expected in view of the microscopic size of the lesions. Cattaneo and Vercillo's (7) patients with pulmonary involvement were discovered chiefly by roentgenographic examination, and clinical and autopsy mani-

festations in the same patients were not described; finally, Mazzei and Masnatta (10) recently described four cases of acute leukemia in which clinical and roentgenographic abnormalities were attributed to the leukemia without autopsy confirmation of the specific findings.

The present writers are convinced from their own study, as well as from those reviewed above, that it is essential to correlate the roentgenographic abnormality with the autopsy findings in each patient with leukemia who develops abnormal pulmonary shadows. It is then possible to distinguish between leukemic involvement of clinical significance and that limited to histologic abnormality, a determination of great importance to the clinician and, to our knowledge, one not previously alluded to in the literature. When this was done in the present study, it was found that, although the total incidence of infiltration was 27 per cent, the involvement of clinical significance was 7 per cent. There was a slight difference between acute and chronic leukemia in this respect, the figures being 4 and 10 per cent, respectively. However, the total number of cases was too small to be sure of this distinction. Perhaps more significance can be attached to the difference between chronic lymphatic leukemia, on the one hand, and chronic myelogenous leukemia as well as all other forms, on the other: 17 per cent of the patients with chronic lymphatic leukemia had pulmonary involvement of clinical and roentgenographic significance and as, in addition, the problem of differential diagnosis of pleural effusion occurred predominantly in these patients, it is clear that leukemic pulmonary involvement is both a common and clinically significant manifestation of chronic lymphatic leukemia.

In the present study, when diffuse pulmonary infiltrations appeared during the course of leukemia, leukemic infiltrations per se were responsible in less than 10 per cent of the instances (table 3). The largest single cause was infection of various types; and the importance of nonspecific pulmonary infection should be emphasized, as one-third of the patients with diffuse pulmonary infiltrations had bronchopneumonia with or without areas of abscess formation at the time of death. The infections were often overwhelming and were frequently the immediate cause of death in these patients.

In addition, these infections did not respond to antimicrobial therapy in the usual manner, thus adding further to the difficulty in the differential diagnosis between leukemia and infection. For example, the roentgenogram in figure 6 is that of a 60-year-old man with chronic lymphatic leukemia who had been on prolonged adrenal corticosteroid therapy. He developed what appeared clinically to be overwhelming diffuse pulmonary infection which, however, failed to respond to antimicrobial therapy, so that the possibility of leukemic infiltration was raised. At autopsy the sections revealed multiple areas of pneumonia and abscess formation with meager cellular response and some organization of the inflammatory exudate. It should be emphasized that the reverse situation also occurred; that is, that in one patient in this series diffuse pulmonary infiltrations which showed considerable spontaneous resolution at autopsy were due to a minor degree of diffuse leukemic involvement. No evidence of infection was found. It is probable that the roentgenographic abnormality was due to leukemia which had partially resolved spontaneously. Nathan and Sanders (6) were of the opinion that the pulmonary lesion causing alveolar-capillary block in their patient with acute leukemia was probably leukemic infiltration because of the favorable response to amethopterin, although there was no histologic proof. This was probably correct but, in general, the disappearance of roentgenographic shadows may not be related to any specific therapy, nor does the failure of resolution of any specific pulmonary shadow imply anything with respect to its etiology.

When pleural disease was found by roentgenogram (table 4), leukemia was a frequent (45 per cent) cause, unlike the situation in the parenchymal infiltration group. However, the frequency of infection is also noteworthy, as this was the cause of the pleurisy in 4 of the 11 patients with pleural disease. Again, in contrast to the parenchymal disease group in which pyogenic infection was much more frequent than tuberculosis or fungal disease, the cause of the pleural disease in 3 of the 4 patients with infection was tuberculosis. This had been diagnosed during life in one of the patients, but had not been suspected in the other 2. Two of these patients had chronic lymphatic leukemia; the third, the chronic myelogenous form. It is thus apparent that, when pleural effusion develops during the

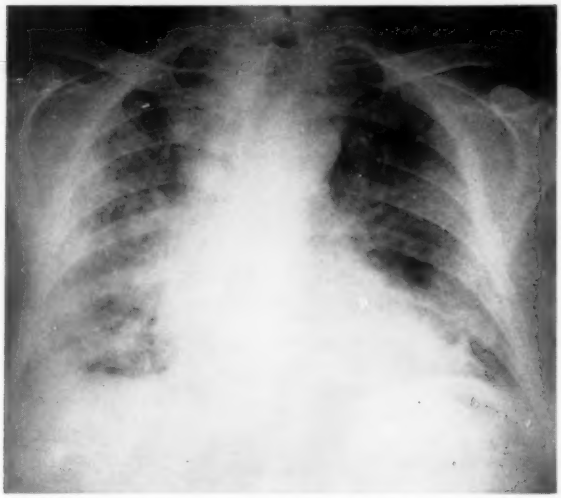

FIG. 6. Chest roentgenogram, antero-posterior view. Diffuse bilateral infiltration due to bronchopneumonia.

course of chronic leukemia, especially the lymphatic variety, the clinician must be alert to the possibility of a tuberculous etiology and institute appropriate diagnostic measures before the diagnosis of the other frequent cause of pleural effusion, leukemia itself, is assumed.

The first years encompassed by the present study (1946–1950) were prior to the period in which adrenal steroid therapy and the newer antimicrobial preparations had entered into common use in the management of patients with leukemia. It is thus possible that the frequency of pyogenic infections will decrease and the frequency of tuberculosis and fungal infections will increase in future years, as has already been reported by Zimmerman (11).

The possibility that pulmonary vascular obstruction by masses of leukemic cells can cause parenchymal changes is discussed in the literature. Fiessinger and Fauvet (12) reported a case of chronic myelogenous leukemia in which the presence of rales and many small round cavities noted roentgenographically suggested widespread pulmonary involvement. Autopsy revealed that the small pulmonary vessels were full of leukemic cells and that the adjacent lung contained nodular aggregates of leukemic cells with central areas of liquefaction that they attributed to proteolytic enzymes liberated by these cells. Except for lesions which were interpreted as lung abscesses, no similar parenchymal lesions were noted in the present series; however, small pulmonary vessels choked with leukemic cells were an extremely common finding and could not be correlated with other parenchymal abnormalities of an infectious, infarctive, or infiltrative nature.

Joachim and Loewe (13) described a patient with diffuse migrating pulmonary infiltrations suggestive of pulmonary infarctions who at autopsy proved to have atypical acute myeloid leukemia. The pulmonary lesions at autopsy were nodular, but, nevertheless, histologically were suggestive of organized pulmonary infarctions. The writers attributed these infarctions to occlusion of pulmonary blood vessels by myeloid cells. No similar clinical case was present in the present series; but pulmonary infarctions were noted in 3 patients. In none of these 3 was cardiac disease or peripheral venous thrombosis known to be present. In one of these patients the infarct was surrounded by infiltration with leukemic cells, apparently resembling the pathology described by Joachim and Loewe (13), although in an earlier stage. No definite vascular occlusions could be identified in 2 patients. The third patient had no clinical manifestations to suggest infarction, although a large infarcted

area of the lung was found at autopsy. The blood vessels in this patient were full of typical ante-mortem thrombi which contained large numbers of leukemic cells. It is impossible on histologic grounds to decide whether this lesion represents an embolus or a thrombosis *in situ*. It was also not possible to distinguish between a perfectly ordinary thrombus in which leukemic cells have been trapped and a thrombus due to the presence of leukemic cells. However, as blood vessels full of leukemic cells without associated thrombi were seen so frequently in this series, the present writers believe that the thrombosis was most likely unrelated to the leukemic cells in the vessels. Therefore, they have been unable to substantiate the presence of pulmonary abnormalities due to simple occlusion of pulmonary vessels by leukemic cells—the so-called "tumor thrombi" of other writers—in this series.

Summary

Microscopic infiltration of the lung and pleura by leukemic cells was found in 27 per cent of a series of 109 cases of leukemia examined at autopsy. The infiltration was of clinical significance in 7 per cent of the cases. Various aspects of the differential diagnosis of pulmonary infiltration and pleural effusion in leukemic patients are discussed.

Sumario

Invasión Pulmonar en la Leucemia

Se observó infiltración microscópica del pulmón y de la pleura por células leucémicas en 27 por ciento de una serie de 109 casos de leucemina examinados en la autopsia. La infiltración poseía importancia clínica en 7 por ciento de los casos. Discútense varios aspectos del diagnóstico diferencial de la infiltración pulmonar y del derrame pleural en los leucémicos.

Resume

Complication pulmonaire dans la leucémie

Une infiltration microscopique du poumon et de la plèvre par des cellules leucémiques a été trouvée dans 27% de 109 cas de leucémie examinés à l'autopsie. Cette infiltration s'était manifestée cliniquement dans 7% de ces cas. Les différents aspects du diagnostic différentiel de l'infiltration pulmonaire et de l'épanchement pleural chez des malades atteints de leucémie sont discutés.

Acknowledgment

The writers wish to express their deep gratitude to Dr. Benjamin S. Gordon and Dr. Samuel Melamed of the Laboratory Service for their helpful discussions of some of the pathologic and hematologic aspects, respectively, of this study; and to the staff of the Medical Illustration Service for the preparation of the illustrations.

REFERENCES

(1) Green, R. A., Nichols, N. J., and King, E. J.: Alveolar-capillary block due to leukemic infiltration of the lung, Am. Rev. Resp. Dis., 1959, *80*, 895.

(2) Rabin, C.: Radiology of the chest, in *Diagnostic Radiology*, Vol. 1, R. Golden, Williams & Wilkins Co., Baltimore, 1952, p. 182 Q.

(3) Schinz, H. R., Baensch, W. E., Friedl, E., and Vehlinger, E.: Roentgen Diagnostics, Vol. 3, Thorax, Grune & Stratton, New York, 1953, p. 2505.

(4) Vieta, J. O., and Craver, L. F.: Intrathoracic manifestations of lymphomatoid diseases, Radiology, 1941, *37*, 138.

(5) Falconer, E. H., and Leonard, M. E.: Pulmonary involvement in lymphosarcoma and lymphatic leukemia, Am. J. M. Sc., 1938, *195*, 294.

(6) Nathan, D. J., and Sanders, M.: Manifestations of acute leukemia in the parenchyma of the lung, New England J. Med., 1955, *252*, 797.

(7) Cattaneo, I., and Vercillo, L.: Criteria for the evaluation of pleuro-broncho-pulmonary location of leukemic disease, Haematologica, 1957, *42*, 749.

(8) Liebow, A. A.: Tumors of the lower respiratory tract, in *Atlas of Tumor Pathology*, Section V, Fascicle 17, Armed Forces Institute of Pathology, Washington, D. C. 1952, p. 170.

(9) Kirklin, B. R., and Hefke, H. W.: Roentgenologic study of intrathoracic lymphoblastoma, Am. J. Roentgenol., 1931, *26*, 681.

(10) Mazzei, E. S., and Masnatta, G.: Pleuropulmonary aspects in the course of acute leukemia, Presse méd., 1957, *65*, 1689.

(11) Zimmerman, L. E.: Fatal fungus infections complicating other diseases, Am. J. Clin. Path., 1955, *25*, 46.

(12) Fiessinger, N., and Fauvet, J.: Le poumon leucémique, Presse méd., 1941, *49*, 449.

(13) Joachim, H., and Loewe, L.: Atypical acute myeloid leukemia with unusual pulmonary manifestations, Am. J. M. Sc., 1927, *174*, 215.

STUDIES OF THYROID FUNCTION IN PATIENTS TREATED WITH PARA-AMINOSALICYLIC ACID AND ISONIAZID[1, 2]

EDWARD SEINFELD AND PAUL STARR[3]
With the technical assistance of Margaret Simpson

(Received for publication March 6, 1959)

INTRODUCTION

The antituberculous drugs, para-aminosalicylic acid (PAS) and isoniazid, are known to have an anti-thyroid effect. A few cases of goiter and hypothyroidism due to PAS were reported by Bergquist and de Marc (1), Clausen and Kjerulf-Jensen (2), Komrower (3), Librach (4), Davies and Galbraith (5), Brinkman and Coates (6), and Macgregor and Sommer (7). Angel and associates (8) reported the effect of certain antituberculous drugs upon the iodine metabolism of surviving thyroid tissue slices in culture media. Para-aminosalicylic acid had a strong anti-thyroid action. Isoniazid also had a definite anti-thyroid action. Streptomycin and dihydrostreptomycin had no anti-thyroid effect. Beattie and Chambers (9) demonstrated that the administration of PAS to albino rats for fifteen weeks resulted in reactive hyperplasia of the thyroid gland and an increase of the I^{131} uptake. The thyroid gland structure of albino rats treated for twenty-four weeks showed evidence of "exhaustion." The I^{131} uptake fell to control levels. The gland structure of these rats reverted to normal after PAS had been discontinued for two weeks.

The present study was undertaken to determine the extent of alteration of thyroid function among patients who had been taking daily 12 gm. of PAS and 150 to 300 mg. of isoniazid for six months or more.

METHODS

Patients at two hospitals were studied: at The City of Hope Medical Center, Duarte, California; and the Veterans Administration Hospital, San Fernando, California.

Serum-protein-bound iodine and cholesterol determinations were performed at the Thyroid Research Laboratory of the University of Southern California Medical School. The I^{131} thyroid uptake studies were performed twenty-four-hours after the ingestion of 10 mc. of radioactive iodine. The normal range of serum-protein-bound iodine is 4 to 8 γ per 100 ml.; blood cholesterol, 130 to 250 mg. per 100 ml.; twenty-four-hour I^{131} thyroid uptake, 15 to 40 per cent.

The data of control and treated patients at each hospital are tabulated separately for two reasons: (*1*) At the Veterans Administration Hospital, the PAS had been administered as liquid or tablet sodium PAS. Calcium PAS granules were prescribed for patients who could not tolerate the liquid or tablet PAS. The outpatient groups at the Veterans Hospital had been provided with coated calcium PAS granules exclusively. At the City of Hope Medical Center, sodium PAS tablets were almost uniformly provided and calcium PAS granules, rarely. (*2*) The I^{131} uptakes were taken with the different scintillation counters available at each hospital.

RESULTS

No patients with myxedema or goiter were discovered at either hospital.

At the City of Hope, I^{131} uptake of the control subjects, i.e., 6 newly admitted patients not on drugs, averaged 23.9 per cent; their serum-protein-bound iodine averaged 5.6 γ per 100 ml., ranging between 4.8 and 6 γ per 100 ml.; their serum cholesterol averaged 254 mg. per 100 ml. ranging between 225 and 330 mg. per 100 ml. (table 1). Among a group of 13 men and women who had been receiving PAS and isoniazid for more than six months, the I^{131} uptakes averaged 73.2 per cent when performed three days after PAS was discontinued. When repeated after an interval of three months without the withdrawal of PAS, they averaged 48.8 per cent. The serum-protein-bound iodine of the 13 treated patients averaged 5.4 γ per 100 ml., ranging between 4.0 and 8.9 γ per 100 ml.; the serum cholesetrol averaged 274.6 mg. per 100 ml., ranging between 170 and 350 mg. per 100 ml. (table 2).

At the San Fernando Veterans Hospital, 98

[1] From the Department of Medicine, University of California School of Medicine, Los Angeles, California.

[2] Supported by a grant from the Los Angeles Tuberculosis Association.

[3] Present address: 144 East Artesia Street, Pomona, California.

TABLE 1

STUDIES ON PATIENTS WHO HAD NOT HAD CHEMOTHERAPY AT THE CITY OF HOPE MEDICAL CENTER, DUARTE

Patient	Per Cent Uptake on I^{131}	Protein-bound Iodine	Cholesterol
		γ	
M.Q.	29.0	5.8	330
I.A.	25.0	6.0	225
I.S.	13.1	5.1	240
D.M.	24.1	6.0	240
W.S.	25.3	5.8	250
M.F.	27.0	4.8	235
Average	23.9	5.6	254

patients were studied. Twenty-six of these were control subjects, i.e., patients not yet receiving the drugs; 37 were inpatients and 35, outpatients. Except for the control subjects, the patients had received PAS and isoniazid for at least six months (table 3).

The control subjects varied in age from twenty-seven to eighty years, averaging fifty years. The percentage of I^{131} uptake varied between 3.4 and 33.1 per cent, averaging 16.8 per cent. The serum-protein-bound iodine averaged 6.1 γ per 100 ml. with a variation between 2.6 and 8.4 γ per 100 ml. Except for one control subject with a protein-bound-iodine value equal to 2.6 γ per 100 ml., the lowest protein-bound-iodine value equalled 4.5 γ per 100 ml. The serum cholesterol averaged 307 mg. per 100 ml. and ranged between 185 and 400 mg. per 100 ml.

The treated patients have been divided into inpatient and outpatient groups. These groups were further subdivided according to the number of months during which they had received PAS and isoniazid: *Group I* consisted of the control subjects. The members of *Group II* had received medication from six to twenty-four months. The members of *Group III* had been treated for twenty-five to thirty-six months, and in *Group IV* medication had been given for thirty-seven to forty-eight months; in *Group V*, for forty-nine months or more. The results of the I^{131} uptake,

TABLE 2

STUDIES ON PATIENTS WHO HAD BEEN ON ISONIAZID-PAS SIX MONTHS OR MORE AT THE CITY OF HOPE MEDICAL CENTER, DUARTE

Patient	Per Cent Uptake on PAS	Per Cent Uptake off PAS	Protein-bound Iodine	Cholesterol
			γ	
C.J.	42.4	71.4	8.9	250
H.M.	50.6	80.0	4.1	290
J.K.	83.5	102.8	6.1	240
R.R.	42.0	46.0	6.1	285
R.B.	59.3	84.3	4.7	170
M.C.	39.7	77.9	5.0	230
J.T.	45.6	61.8	5.7	350
O.M.	46.5	65.4	4.9	290
A.F.	43.0	79.0	4.7	280
J.H.	50.0	90.8	4.0	310
R.C.	50.4	72.4	4.8	295
J.L.	40.7	53.0	6.5	290
M.T.	41.3	66.6	4.8	290
Average	48.8	73.2	5.4	274.6

TABLE 3

STUDIES ON PATIENTS WHO HAD BEEN ON ISONIAZID-PAS SIX MONTHS OR MORE AT THE U. S. VETERANS ADMINISTRATION HOSPITAL, SAN FERNANDO, CALIFORNIA

Tabulated Summary

Group	Months on Drugs		Average Age	Average Per Cent I^{131} Uptake	Average Protein-bound Iodine	Average Inorganic Iodine	Average Cholesterol
					γ		
1	No isoniazid-PAS	Inpatients	50 (26)	16.8 (26)	6.1 (24)	5 (24)	307 (24)
2	6 to 24	Inpatients	47 (14)	17.2 (14)	4.3 (11)	5 (14)	259 (14)
		Outpatients	39 (5)	27.3 (3)	7.3 (5)	5 (5)	227 (14)
3	25 to 36	Inpatients	50 (7)	18.1 (9)	4.6 (9)	4 (9)	271 (5)
		Outpatients	37 (10)	33.3 (2)	7.0 (10)	4 (10)	295 (9)
4	37 to 48	Inpatients	50 (7)	15.9 (7)	5.0 (7)	5 (7)	300 (6)
		Outpatients	43 (13)	33.1 (6)	6.3 (13)	4 (13)	277 (10)
5	49 and more	Inpatients	52 (7)	19.1 (7)	4.9 (7)	4 (6)	262 (6)
		Outpatients	41 (7)		6.5 (7)	4 (7)	281 (5)

( ) Number included in average

the protein-bound-iodine, and cholesterol determinations are tabulated in table 3.

The I^{131} uptake averages among all of the inpatient groups approximated the average I^{131} uptake of the control subjects; whereas the average I^{131} uptake of the treated groups of outpatients was a high normal, or almost twice the average uptake of the control groups.

A very significant finding is the consistently low normal average protein-bound-iodine values in all inpatients, compared with the average protein-bound-iodine values of the outpatients. However, examination of individual patients revealed that, among the inpatients, there were 12 of 34 whose protein-bound-iodine concentrations were less than 4 γ per 100 ml. Of these, 6 patients were in *Group II*, 3 patients in *Group III*, one patient in *Group IV*, and 2 patients in *Group V*.

Discussion

The thyroid iodine "trap" is that capacity of the thyroid gland to combine with inorganic iodine. This capacity is increased in hyperthyroidism and by those factors which disturb the balance of the thyroid-pituitary axis. Anterior pituitary thyrotropic hormone or thyroid-stimulating hormone levels are balanced by levels of circulating thyroxine. A tendency for a reduction in thyroxine production causes an increase in thyroid-stimulating hormone production with increased stimulation of the thyroid gland. Under normal circumstances inorganic iodine is rapidly converted to organic iodine by the thyroid. The blocking of this action by thiourea, mercaptoimidazole, and aminophenol compounds results in reduction of the thyroid hormone blood levels. The increased thyroid-stimulating hormone response stimulates increased metabolic activity and hyperplasia of the thyroid gland. Although thyroxine production may be effectively blocked, the ability to concentrate inorganic iodine remains unimpaired or may be enhanced.

The anti-thyroid effect of isoniazid-PAS is probably due predominantly to the PAS, which has a direct anti-thyroid effect only slightly less than thiouracil. There have been no reports of isoniazid's being goitrogenic in animals or humans, although an anti-thyroid effect has been demonstrated in rat thyroid tissue slices in culture media.

The high I^{131} uptake of the patients of the City of Hope Medical Center to whom isoniazid-PAS had been prescribed for six months or more suggests that the iodide trap had been expanded. Further enhancement of iodide trapping was demonstrated when the I^{131} uptake was repeated after the PAS had been discontinued for three days. The blocking action of PAS (and isoniazid) on the iodide-trapping mechanism probably provoked increased thyroid-stimulating hormone production, increased thyroid stimulation, and expansion of the iodide trap. As the serum-protein-bound iodine of the treated patients and controls was essentially the same, 5.4 γ per 100 ml. and 5.6 γ per 100 ml., thyroxine production had been unaffected by the treatment with PAS (and isoniazid) at the City of Hope Medical Center.

Similar results were obtained among the outpatients at the San Fernando Veterans Administration Hospital. While the patients were receiving PAS (and isoniazid), the I^{131} uptakes were considerably higher than those of the control subjects, whereas serum-protein-bound iodine concentrations approximated the control values. The I^{131} uptakes among the inpatients approximated the control values, but the serum-protein-bound iodine concentrations were significantly lower than in the controls in each of the four groups. Apparently, prolonged administration of isoniazid-PAS and probably higher peak concentrations had resulted in impairment of thyroxine production and of the compensatory mechanism which had expanded the thyroid iodide trap of the other series of patients.

The marked effect of PAS on the thyroidal uptake of I^{131} among the treated patients at the City of Hope and the outpatients at the San Fernando Veterans Hospital may be compared with the elevation in the average I^{131} uptake among the albino rats to which PAS had been administered for fifteen weeks by Beattie and Chambers (9).

The average I^{131} uptake of the groups of inpatients at the San Fernando Veterans Hospital approximated the control average, as did the average of I^{131} uptake of the albino rats to which PAS had been administered by Beattie and Chambers for twenty-four weeks.

The discrepancy in the averages of I^{131} uptakes of the inpatients at the Veterans Hospital compared with those of the other groups of patients may be due to a variation in peak concentrations of PAS. Sodium PAS is administered to the inpatients at the Veterans Hospital in liquid or

tablet form under close surveillance, whereas calcium PAS coated granules, giving lower peak concentrations, are distributed to the veteran outpatients.

Summary

Prolonged ingestion of PAS and isoniazid for six months or more may indirectly stimulate the expansion of the thyroid iodide trapping mechanism, thereby increasing I^{131} uptake above average normal concentrations. The PAS is presumably wholly or predominantly responsible for the effect. The administration of isoniazid-PAS also inhibits iodine trapping by the thyroid gland, as manifested by the marked increase in thyroidal iodine uptake three days after PAS was discontinued. Prolonged administration of isoniazid and PAS may depress thyroxine production with no overt signs of goiter or hypothyroidism. All tuberculous patients on prolonged treatment with isoniazid-PAS should be observed for possible subclinical hypothyroidism.

Sumario

Estudios de la Función Tiroidea en Enfermos Tratados con Acido Para-Aminosalicílico e Isoniacida

La prolongada ingestión de PAS e isoniacida durante seis meses o más puede excitar indirectamente la expansión del mecanismo atrapador del yoduro tiroideo, elevando así la toma de I^{131} a límites superiores a los promedios normales. El PAS es presuntamente la causa absoluta o predominante de este efecto. La administración de isoniacida-PAS también inhibe el atrape de yodo por el tiroides, según manifestó el notable aumento en la toma tiroidea de yodo a los tres días de discontinuar el PAS. La prolongada administración de isoniacida y PAS puede deprimir la producción de tiroxina sin signos evidentes de bocio o hipotiroidismo. A todos los tuberculosos a prolongado tratamiento con isoniacida-PAS hay que observarlos en busca de posible hipotiroidismo subclínico.

Resume

Études de la fonction thyroidienne chez les malades traités par l'acide para-aminosalicylique et l'isoniazide

L'ingestion prolongée de PAS et d'isoniazide pendant six mois ou plus, peut stimuler indirectement la rétention d'iodure par la thyroide, et augmenter ainsi l'absorption de I^{131} au delà des taux normaux. Ce résultat est probablement dû en partie ou entièrement au PAS. L'administration d'isoniazide-PAS inhibe aussi la rétention d'iode par la glande ainsi que le montre l'augmentation marquée de l'absorption d'iode par la thyroide 3 jours après l'arrêt du traitement par PAS. Une administration prolongée d'isoniazide-PAS peut entraîner la diminution de la production de thyroxine sans apparition de signe de goître ou d'hypothyroidisme. Tous les tuberculeux soumis à un traitement prolongé par isoniazide-PAS devraient être suivis afin de déceler la possibilité d'un hypothyroidisme latent.

Acknowledgments

The writers wish to acknowledge the cooperation of Dr. Leo Tepper, Medical Director, Chest Hospital, City of Hope Medical Center; Dr. David Salkin, Chief of Professional Services, U. S. Veterans Administration Hospital; Dr. Sanford Lawrence, Staff Physician, U. S. Veterans Administration Hospital, San Fernando, California.

REFERENCES

(1) Bergqvist, N., and de Mare, O.: Hypothyroid goiter and cerebral edema due to Conteben intoxication: Fatal case, Nord. med., 1951, *45*, 474.

(2) Clausen, K. H., and Kjerulf-Jensen, K.: Artificial myxedema during administration of para-aminosalicylic acid, Nord. med., 1951, *45*, 475.

(3) Komrower, G. M.: Case of myxoedema developing during para-aminosalicylic acid therapy, Brit. M. J., 1951, *2*, 1193.

(4) Librach, I. M.: Antithyroid action of P.A.S., Lancet, 1952, *2*, 538.

(5) Davies, H. T., and Galbraith, J. J. B.: Goitre and hypothyroidism developing during treatment with P.A.S., Brit. M. J., 1953, *1*, 1261.

(6) Brinkman, G. L., and Coates, E. O.: Goitrogenic effect of para-aminosalicylic acid during therapy of pulmonary tuberculosis: Report of 11 cases, Am. Rev. Tuberc., 1954, *69*, 458.

(7) Macgregor, A., and Somner, A.: Antithyroid action of para-aminosalicylic acid, Lancet, 1954, *2*, 931.

(8) Angel, R. W., Mayer, S. W., and Morton, M. E.: Direct antithyroid action of para-aminosalicylic acid and isoniazid, Am. Rev. Tuberc., 1955, *71*, 889.

(9) Beattie, J., and Chambers, R. D.: The antithyroid action of para-aminosalicylic acid, J. Endocrinol, 1953, *10*, 65.

VIRULENCE OF THE TUBERCLE BACILLUS[1,2]

III. Effect of Penicillin upon the Growth of Virulent and Avirulent Bacilli

CHARLES T. UYEDA AND SIDNEY RAFFEL

(Received for publication July 15, 1959)

INTRODUCTION

The exact nature of the factor responsible for the virulence of *Mycobacterium tuberculosis* has not been convincingly explained (1–3). Certain morphologic (4), biochemical (5–7), and toxic (8–10) characteristics have been associated with the ability of mammalian tubercle bacilli to cause infections and to disrupt cells in tissue culture (11–14). But this information so far has not revealed what role, if any, these correlative properties may exert in determining virulence. It may be, of course, that virulence in the case of this bacterium is "passive"; that disease-producing organisms have no special property harmful to the host's defenses because the host has no defenses; and that survival of virulent strains in the tissues depends upon a simple metabolic ability to meet the cultural conditions imposed by the tissues. Yet, it is difficult to believe that the differences noted between virulent and avirulent strains are simply coincidental, without meaning in regard to pathogenic properties.

Fenner and Leach (15) found that, regardless of virulence, most strains of mammalian mycobacteria grow at about the same rate on the usual synthetic or egg media if adequately aerated. However, work in this laboratory (16, 17) has indicated that, under the adverse environmental condition imposed by low oxygen tension, the growth rate is altered, the virulent strains outgrowing the avirulent. This observation has since been confirmed (18). Possibly related to this observation is that reported by Mackaness, Smith, and Wells (19) to the effect that attenuated bacilli multiply more slowly than do virulent ones within normal monocytes in tissue culture, with a much longer lag period. This fact may depend upon features within the monocyte other than low oxygen tension, for the present writers have found (3) something similar in culture media. If virulent and avirulent strains are repeatedly subcultured on a particular medium and then transferred to a new one, the virulent strains adapt more readily to growth on the new medium than do the avirulent bacilli. It is suggestive that strains which have greater adaptability to new or to disadvantageous environments may have greater survival potential in the environment provided by a host.

The present report deals with the differential effect of another factor, penicillin, which may be considered a disadvantageous environmental one upon the growth of virulent, attenuated, and avirulent tubercle bacilli.

MATERIAL AND METHODS

Eleven laboratory strains of *M. tuberculosis* were employed in this investigation. These include the virulent human variants designated as H37Rv, Cutter, Campbell, 88, and the virulent bovine strain Ravenel; the attenuated human strain R1Rv and the attenuated bovine strain BCG; and the avirulent strains H37Ra, H4Ra, JHB6, and JHB16. These strains were maintained on glycerin egg medium. For the present studies, they were transferred to Dubos Tween® liquid medium (20) in which they were repeatedly subcultured. Ten- to thirteen-day-old cultures were employed.

Experiments in which crystalline penicillin G[3] was incorporated in both Dubos oleic-acid–albumin liquid and agar media were set up as follows: The liquid medium was used in 5-ml. quantities incorporating the desired concentrations of penicillin, and tubes were inoculated with 0.5 ml. of a 1:10,000 dilution in saline of the inoculating suspension which had first been brought to an optical density reading of 50 in the Klett-Summerson colorimeter (green filter #54). For the solid medium test, 6-cm. diameter Petri dishes were layered with 5 ml. of agar containing penicillin, and 0.1 ml. of the inoculating suspension was spread evenly over the surface of the agar with the aid of 0.3 ml. of saline. The plates were incubated overnight in the upright position

[1] From the Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California.

[2] This work has been supported by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the California Tuberculosis & Health Association; and the Harold Guyon Trimble Memorial Fund.

[3] Manufactured by The Upjohn Company.

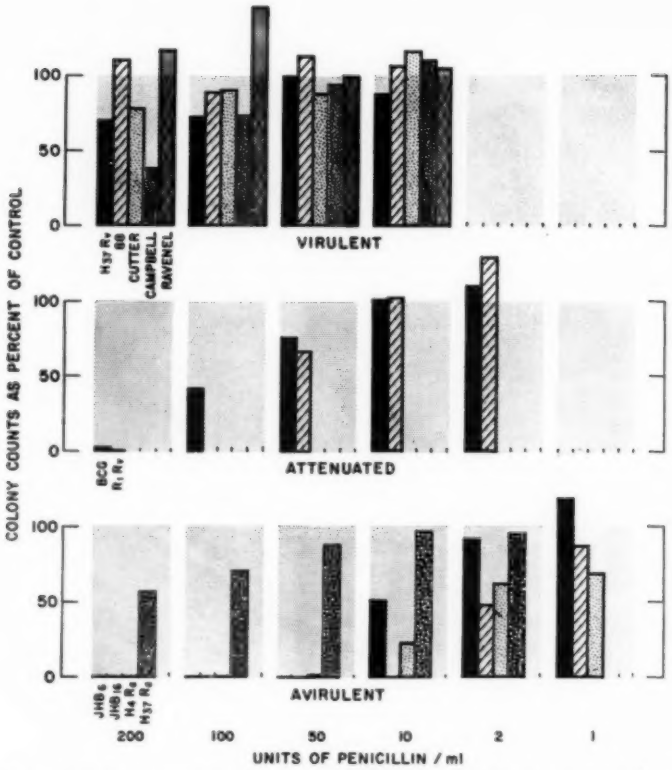

FIG. 1. Effects of various concentrations of penicillin upon virulent, attenuated, and avirulent strains of *M. tuberculosis*.

and were then sealed with Parafilm® and inverted for incubation.

Tubes and plates were examined at weekly intervals for six weeks; the former for turbidity, the latter for colony counts. Tubes and plates in all cases were run in triplicate.

RESULTS

The results of all experiments carried out are presented in figures 1, 2, and 3. In figure 1, the results of various experiments with each strain have been combined for the presentation of average figures for growth at six weeks under the influence of various concentrations of penicillin on solid medium, and, although the evaluations of growth were made simply by visual comparison of tubes for turbidity, the results coincided well with those obtained from the quantitative counts of bacterial units developing on agar.

Four of the 5 virulent strains were quite resistant to 200 and 100 units of penicillin per ml. of medium, and were virtually completely resistant to 50 units and lesser quantities. Of these 4 strains, one, the Ravenel, appeared to be completely resistant to all concentrations; in fact, there is a suggestion here that the penicillin may stimulate growth to some extent. One of the 5 strains, the Campbell, a human variant of *M. tuberculosis* received from Dr. Steenken of the Trudeau Sanatorium several years ago and since kept in culture on egg glycerin medium, showed less resistance than the other 4 in the 200- and 100-unit tests on agar.

The two attenuated strains, BCG and R1Rv, both showed an impaired ability to resist penicillin at the higher concentrations as compared with the virulent strains. Both were almost completely inhibited by 200 units and relatively inhibited even by 50 units. This effect was not as apparent in the liquid medium; in the presence of 100 units of penicillin per ml., these

strains developed as well as in the absence of the drug.

Of the 4 avirulent strains one, H37Ra, behaved in a fashion intermediate between that of the virulent and the attenuated strains. It showed some resistance even to 200 units per ml. The other 3 avirulent strains were obviously much more strongly inhibited even by lower concentrations; in fact, with 2 of these strains, JHB16 and H4Ra, there was evidence of growth suppression in the presence of two and even perhaps of one unit of the drug. On the basis of these results, the writers have since refrained from using any penicillin in cultural studies employing either attenuated or avirulent bacilli, as in tissue cultures.

It is interesting that H37Ra, the most penicillin-resistant strain, is the only member of this group which showed cording, a fact that has been reported by others in the past. However, tests in guinea pigs have always been completely negative in revealing any tendency to cause infection.

In figure 2, the two-week growth figures for the three groups of strains, virulent, attenuated, and avirulent, have been averaged, and similarly combined data for the six-week growth period

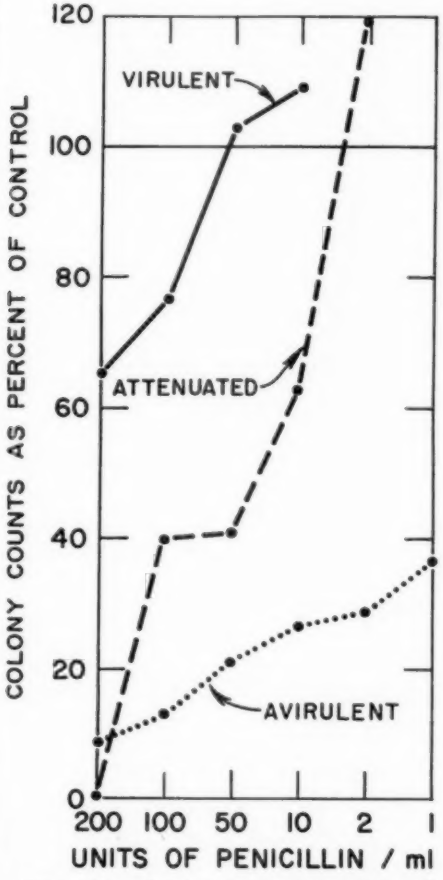

FIG. 2. Growth of virulent, attenuated, and avirulent tubercle bacilli under the influence of various concentrations of penicillin after two weeks.

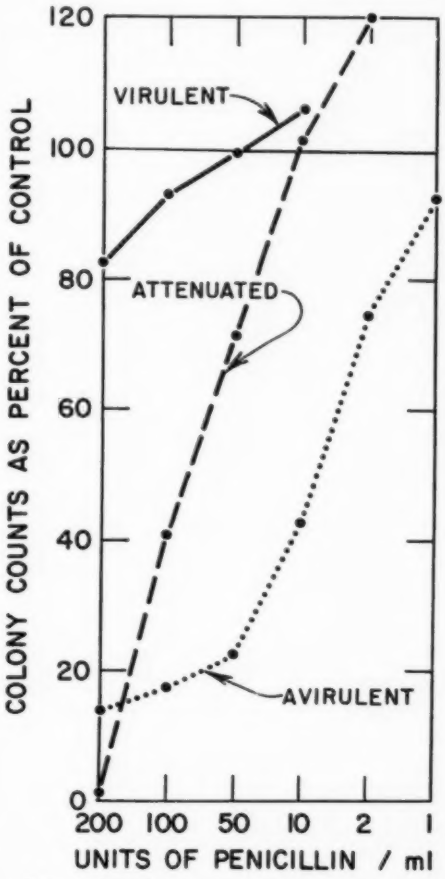

FIG. 3. Growth of virulent, attenuated, and avirulent tubercle bacilli under the influence of various concentrations of penicillin after six weeks.

are shown in figure 3. The differences in susceptibilities are more apparent at the earlier time, possibly because of a progressive deterioration of penicillin in the medium, but the variable influence of the drug upon strains of differing degrees of virulence reveals itself quite clearly in both of these graphs.

The question arose whether the small amount of Tween 80 present in the inoculum used for these tests may have influenced susceptibility to penicillin, in view of the findings of Kirby and Dubos (21) that this detergent greatly increases the vulnerability of virulent organisms to the drug. Unpublished data accumulated by Higashi (22) revealed that this was not so; simultaneous experiments utilizing organisms removed from the surface of solid medium in one set of tests, and from Tween broth cultures in the other, gave similar results.

Discussion

Previous reports dealing with the extent of inhibitory activity of penicillin upon *M. tuberculosis* have been concerned with virulent strains, and it is well known that this drug is ineffective for practical therapeutic purposes, although it does have inhibitory properties in sufficiently high concentrations. A number of investigators, including Abbott (23), Fallin and associates (24), and Ungar and Muggleston (25), found no significant inhibitory effect upon virulent bacilli with concentrations of penicillin of less than 100 units per ml., although Hand (26) reported that smaller concentrations, between 20 and 80 units, may suppress the growth of bacilli obtained directly from patients. Kirby and Dubos (21), along with a majority of other investigators, found that virulent strains were resistant to 100 units per ml., but that in a Tween-containing medium as little as 1 unit per ml. was sufficient for inhibition.

The present report confirms the general tenor of previous findings with virulent organisms and is submitted mainly because of the observation that attenuated and avirulent mycobacteria have a greater degree of susceptibility to penicillin than do virulent strains. One of the 5 virulent organisms behaved like the attenuated strains, while one of the 4 avirulent strains (H37Ra) acted in a fashion intermediate between that of the virulent and the attenuated. It acted so in other respects also, including the tendency to form serpentine colonies.

This correlation may be purely fortuitous, without any direct relationship of factors determining virulence to those determining drug susceptibility. The differences, for example, could be manifestations of variations in the physical structures of the various bacteria. Thus, the surfaces of virulent strains are reported to be more hydrophobic than those of avirulent ones (27), and this circumstance might alter effective contact of the drug in aqueous solution with susceptible centers of the hydrophobic bacteria, thus rendering them drug resistant. This view is supported by the observation (21) that a surface tension-reducing agent such as Tween 80 greatly increases the susceptibility of virulent tubercle bacilli to penicillin. In the final analysis, however, the hydrophobic character of the surface of the organism may in itself have some causal ability not yet appreciated with respect to virulence.

The observations reported can be added to a number of other characteristics noted as distinguishing mycobacteria of differing degrees of virulence, including cord formation, neutral red reaction, differences in behavior toward oxidation-reduction dyes, and differences in lipoidal contents. At the present time it can only be concluded that here again is a differential characteristic of virulent as opposed to avirulent tubercle bacilli which may be looked upon as another manifestation of greater adaptability of virulent strains to new environmental situations. These observations may also have practical implications for those working with avirulent organisms in cultures or in other experimental situations in which it is desirable to use antimicrobial agents to prevent contamination. Penicillin obviously is not a good drug for this purpose.

Summary

Rather wide differences are described in the susceptibility of virulent, attenuated, and avirulent strains of *M. tuberculosis* to penicillin. Virulent strains in general are highly resistant, attenuated strains show an intermediate level of resistance, and 3 of the 4 avirulent strains worked with are highly susceptible to this drug. The findings have implications for studies using culture medium, tissue cultures, or other experimental situations in which it is desirable to use antimicrobial agents to prevent contamination.

Sumario

Virulencia del Bacilo Tuberculoso: III. Efecto de la Penicilina sobre la Proliferación de Bacilos Virulentos y Avirulentos

Descríbense diferencias algo amplias en la susceptibilidad de las cepas virulentas, atenuadas y avirulentas del *M. tuberculosis* a la penicilina. Las virulentas son en general sumamente resistentes, las atenuadas revelan un tenor intermedio de resistencia y 3 de las 4 cepas avirulentas estudiadas son sumamente susceptibles a dicha droga. Los hallazgos poseen connotaciones para estudios en que figuren medios de cultivo, cultivos de tejido u otras situaciones experimentales en que convenga usar productos antibacterianos para impedir la contaminación.

Resume

Virulence du bacille tuberculeux: Effet de la pénicilline sur la croissance des bacilles virulents et non-virulents

Des différences assez prononcées sont décrites concernant la sensibilité à la penicilline de souches virulentes, atténuées et non-virulentes de *M. tuberculosis*. Les souches virulentes sont en général très résistantes, les souches atténuées présentent un degré intermédiaire de résistance et 3 des 4 souches non-virulentes examinées se sont montrées très sensibles à la penicilline. Ces conclusions présentent un intérêt certain pour les travaux où l'on utilise les milieux de cultures, les cultures sur tissu ou les autres expériences dans lesquelles il est nécessaire d'employer des agents antimicrobiens pour éviter la contamination.

REFERENCES

(1) Dubos, R. J.: Properties and structures of tubercle bacilli concerned in their pathogenicity, in *Mechanisms of Microbial Pathogenicity*, Cambridge University Press, Cambridge, England, 1955, p. 103.

(2) Raffel, S.: Immunity, Hypersensitivity, Serology, Appleton-Century-Crofts, Inc., New York, 1953.

(3) Raffel, S.: Immunopathology of tuberculosis, Am. Rev. Tuberc., 1955, *74* (Supplement, p. 62).

(4) Middlebrook, G., Dubos, R. J., and Pierce, C.: Virulence and morphological characteristics of mammalian tubercle bacilli, J. Exper. Med., 1947, *86*, 175.

(5) Dubos, R. J.: The effects of organic acids on mammalian tubercle bacilli, J. Exper. Med., 1950, *92*, 319.

(6) Geronimus, L., and Birkeland, J. M.: The relationship between the virulence of tubercle bacilli and the vigor of their oxidative attack upon certain substances, Am. Rev. Tuberc., 1951, *64*, 520.

(7) Wilson, F., Kalish, C., and Fish, C.: Use of oxidation-reduction dyes in the determination of virulence of mycobacteria *in vitro*, Am. Rev. Tuberc., 1952, *65*, 187.

(8) Asselineau, J., Damarteau, H., and Lederer, E.: Sur les acides mycoliques de deux souches de *Mycobacterium tuberculosis* var. *hominis* H37Rv and H37Ra: Sur les différentes chimiques entre des souches virulentes et non-virulentes de *Mycobacterium tuberculosis*, Compt. rend. Soc. de biol., 1950, *230*, 142.

(9) Asselineau, J., Bloch, H., and Lederer, E.: A toxic lipid component of the tubercle bacillus ("cord factor"), Am. Rev. Tuberc., 1953, *67*, 853.

(10) Bloch, H., Sorkin, E., and Erlenmeyer, H.: A toxic lipid component of the tubercle bacillus ("cord factor"): I. Isolation from petroleum ether extracts of young bacterial cultures, Am. Rev. Tuberc., 1953, *67*, 629.

(11) Maximow, A.: Étude comparative des cultures de tissus inoculées soit avec le bacille tuberculeux du type bovin soit avec le bacille BCG de Calmette-Guérin, Ann. Inst. Pasteur, 1928, *42*, 225.

(12) Fong, J., Schneider, P., and Elberg, S. S.: Studies on tubercle bacillus-monocyte relationship: I. Quantitative analysis of effect of serum of animals vaccinated with BCG upon bacterium-monocyte system, J. Exper. Med., 1956, *104*, 455.

(13) Fong, J., Schneider, P., and Elberg, S. S.: Studies on tubercle bacillus-monocyte relationship: II. Induction of monocyte degeneration by bacteria and culture filtrate: Specificity of serum and monocyte effects on resistance to degeneration, J. Exper. Med., 1957, *105*, 25.

(14) Berthrong, M., and Hamilton, M. A.: Tissue culture studies on resistance in tuberculosis: I. Normal guinea pig monocytes with tubercle bacilli of different virulence, Am. Rev. Tuberc., 1958, *77*, 436.

(15) Fenner, F., and Leach, R. H.: The growth of mammalian tubercle bacilli in Tween-albumin medium, Am. Rev. Tuberc., 1953, 68, 342.

(16) Heplar, J. Q., Clifton, C. E., Raffel, S., and Futrelle, C. M.: Virulence of the tubercle bacillus: I. Effect of oxygen tension upon respiration of virulent and avirulent bacilli, J. Infect. Dis., 1954, *94*, 90.

(17) Guy, L. R., Raffel, S., and Clifton, C. E.: Virulence of the tubercle bacillus: II. Effect of oxygen tension upon growth of virulent and avirulent bacilli, J. Infect. Dis., 1954, *94*, 99.

(18) Sever, J. L., and Youmans, G. P.: The relation of oxygen tension to virulence of tubercle bacilli and to acquired resistance in tuberculosis, J. Infect. Dis., 1957, *101*, 193.

(19) MACKANESS, G. B., SMITH, N., AND WELLS, A. Q.: The growth of intracellular tubercle bacilli in relation to their virulence, Am. Rev. Tubere., 1954, *69*, 479.

(20) DUBOS, R. J., AND MIDDLEBROOK, G.: Media for tubercle bacilli, Am. Rev. Tubere., 1947, *56*, 334.

(21) KIRBY, W. M. M., AND DUBOS, R. J.: Effect of penicillin on the tubercle bacillus *in vitro*, Proc. Soc. Exper. Biol. & Med., 1947, *66*, 120.

(22) HIGASHI, J.: Work carried out in this laboratory.

(23) ABBOTT, J. N.: Effective use of penicillin to reduce contamination in sputum concentrates to be examined for the tubercle bacilli, Am. J. Pub. Health, 1951, *41*, 287.

(24) FALLIN, J. R., PATNODE, R. A., AND HUDGINS, A. K.: Toxic effects of penicillin on culture of tubercle bacilli, Am. J. Clin. Path., 1952, *22*, 703.

(25) UNGAR, J., AND MUGGLESTON, P.: Effect of penicillin on growth of human type *M. tuberculosis*, J. Path. & Bact., 1946, *58*, 501.

(26) ILAND, C. N.: Effect of penicillin on tubercle bacillus, J. Path. & Bact., 1946, *58*, 495.

(27) LAMANNA, C., AND MALLETTE, M. F.: Basic Bacteriology, Its Biological and Chemical Background, Williams & Wilkins Co., Baltimore, Maryland, 1953, p. 185.

THE DETECTION OF PYRAZINAMIDE-INDUCED LIVER DAMAGE BY SERUM ENZYME DETERMINATIONS[1-3]

JOHN F. MORRISSEY AND RUTH C. RUBIN

(Received for publication August 18, 1958)

Introduction

The initial report of the use of pyrazinamide in the treatment of pulmonary tuberculosis by Yeager, Monroe, and Dessau in 1952 (1) described a significant incidence of liver damage. Subsequent reports by a number of investigators have confirmed this observation. Of 801 cases reviewed from the literature (1–12), 17.5 per cent of the patients developed abnormalities of various liver function tests, and 4.4 per cent became jaundiced. McDermott in 1956 (13) stated that of the first 500 to 600 cases treated with pyrazinamide there were at least 35 cases of hepatitis with 5 deaths. In 1957, the Committee on Therapy of the American Trudeau Society (14) stated that the risk of liver toxicity outweighed the potential therapeutic value of the drug, and the Committee did not believe that the serial determination of liver function tests, including a serum transaminase, would prevent serious toxicity.

In 1956, Wroblewski and LaDue (15) described the case of a tuberculous patient under treatment with pyrazinamide in which the serum glutamic oxalacetic transaminase became elevated three weeks before there was any other clinical or laboratory evidence of hepatic damage. Based on this observation, a clinical study of the patient group under treatment with pyrazinamide was undertaken at the Madison Veterans Administration Hospital. The initial results (16) and the results of others (17, 18) have confirmed the sensitivity of serum enzyme tests in this situation. The present report describes the experience with 95 patients given 102 courses of the drug for a total of 2,316 patient-weeks. Initially in the study, these patients were followed by serial determination of conventional liver function tests and of the serum glutamic oxalacetic transaminase. Later, serial determinations of serum glutamic pyruvic transaminase, serum lactic dehydrogenase, and serum malic dehydrogenase were made.

Plan of Study

The patient group was composed of 86 patients with far advanced pulmonary tuberculosis and 9 patients with moderately advanced disease. Therapy was initiated in 28 courses according to chemotherapy protocols 12B or 18 of the joint Veterans Administration–Armed Forces chemotherapy study. For these patients the companion drug was isoniazid. Thirty-eight courses were given to patients with chronic disease with drug-resistant organisms. The remaining 36 courses were given to provide more certain antimicrobial protection to patients undergoing resectional surgery when actual or potential resistance to the usual chemotherapeutic agents was present. With few exceptions, patients received 3 gm. daily of pyrazinamide in divided dosages.

Bromsulphalein retention and cephalin flocculation tests and the serum glutamic oxalacetic transaminase were performed in all patients before starting therapy. Later, prothrombin time was substituted for the cephalin flocculation test, and the serum glutamic pyruvic transminase and lactic- and malic dehydrogenases were determined. More recently, the alkaline phosphatase test and serum bilirubin have been included. The conventional tests were determined at monthly intervals; serum enzyme determinations were made at biweekly intervals. Tests were determined more frequently when toxicity was present or suspected.

Methods

Bromsulphalein retention was determined by measuring the retention forty-five minutes after a 5.0-mg. per kg. dose. The cephalin flocculation test was performed by the method of Hanger, recording only the twenty-four-hour reading. The prothrombin time was determined by the one-stage Quick method. Alkaline phosphatase was assayed by the Thorngate modification of the King-Armstrong method. Serum bilirubin was measured by the method of Malloy and Evelyn using the Ducci-Watson modification. Thymol turbidity was determined by the method of MacLagan.

[1] From the Veterans Administration Hospital, and the University of Wisconsin Medical School, Madison, Wisconsin.

[2] This research was supported (in part) by research grants E-1612 and E-2014 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service.

[3] Presented in part before the Medical Session, as part of *Section 6B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 21, 1958.

Serum glutamic oxalacetic transaminase was performed by a minor modification of the technique of Karmen, Wroblewski, and LaDue (19, 20). The procedure used was as follows: To 2.0 ml. of 0.1 M potassium phosphate buffer at pH 7.4 were added 0.5 ml. of 0.2 M sodium aspartate solution at pH 7.4, 0.1 ml. of malic dehydrogenase (2,000 units per ml.), 0.1 ml. of reduced diphosphopyridine nucleotide 2.0 mg. per ml., and 0.1 ml. of the patient's serum. This mixture was centrifuged for five minutes at 900 × g, and then incubated for ten minutes at 25°C. The supernatant was quantitatively removed and transferred to a square glass cuvette with a 1.0-cm. light path. Then 0.2 ml. of sodium alpha-keto glutarate solution at pH 7.4 was added with inversion to initiate the reaction. The rate of the reaction was then determined by the decrease in absorbency at 340 mμ using a Beckman B spectrophotometer equipped with a blue sensitive phototube and a blue filter in the shutter assembly. The reaction rate in blanks devoid of serum was so low that it could be neglected under the aforementioned conditions. The reaction rate was measured for two five-minute periods. If the reaction was not linear during this period the determination was repeated, using a smaller volume of serum if a high rate had been encountered. The change in absorbency is produced by the oxidation of reduced diphosphopyridine nucleotide to diphosphopyridine nucleotide. One unit of transaminase is defined as a change in absorbency of 0.001 per minute per ml. of serum. The normal mean was 13 units with a range of normal from 6 to 20 units to include the mean plus- and minus-2 standard deviations. Normal values for the oxalacetic transaminase and for the other enzyme tests were determined in a large group of healthy hospital employees.

The pyruvic transaminase was determined by the method of LaDue, Wroblewski, and Karmen. The procedure was identical to that described for the oxalacetic transaminase except that 0.5 ml. of 0.2 M alanine at pH 7.5 was substituted for the aspartate, and 0.1 ml. of lactic dehydrogenase (8,000 units per ml.) was substituted for the malic dehydrogenase. The mean normal value was 9 units with a range of 2 to 16 units to include the mean plus- and minus-2 standard deviations.

Serum lactic dehydrogenase was determined by the method of Neilands (21) modified in this laboratory for routine clinical use. The procedure employed was as follows: To 1.5 ml. of water were added 1.0 ml. of 0.1 M glycine buffer at pH 10.0, 0.1 ml. of a 0.02 M diphosphopyridine nucleotide solution at pH 7.5, and 0.3 ml. of the patient's serum. The reaction was carried out at 25° C. in a square glass cuvette with a 1.0-cm. light path by the addition with inversion of 0.10 ml. of sodium DL-lactate 0.5 M at pH 7.5. Readings with a Beckman B spectrophotometer were made at one, three, and five minutes at 340 mμ against a distilled water blank. If the reaction was not linear during this period, the determination was repeated with a smaller aliquot of serum if a high rate had been encountered. Again the results are expressed as arbitrary units of absorbency change occurring with the reduction of diphosphopyridine nucleotide to its reduced form. An absorbency change of 0.001 per minute per ml. of serum equals one unit of lactic dehydrogenase activity. The mean normal value was 45 units with a range of 28 to 72 units to include the mean plus- and minus-2 standard deviations.

The method for the malic dehydrogenase is identical to that for the lactic dehydrogenase except that 0.1 ml. of 0.5 sodium-L-malate was used instead of lactate, and 0.5 ml. of serum was used with correspondingly less water. The mean normal is 6.9 units with a range of 3.3 to 10.5 units to include the mean plus- and minus-2 standard deviations.

These enzyme determinations are not difficult to perform if certain precautions are observed. Reagents must be properly refrigerated and kept cold while in use. Serum must be collected atraumatically to avoid hemolysis and must be promptly refrigerated or frozen until the determinations are made. Control of pH and temperature during the reaction must be exact. If these conditions are met, the levels are reliable, reproducible, and often will be found quite constant in a given patient over a period of several months.

TABLE 1

A SUMMARY OF ALL OF THE COURSES OF PYRAZINAMIDE ADMINISTERED DURING THE STUDY

	Number of Courses	Mean Length of Treatment in Weeks
Completed therapy		
Planned termination	26	35.3
Died	2	18.2
Interrupted (medically unsanctioned discharge, failure to return from leave, transfer)	37	20.6
Toxicity		
Serum enzyme elevations	19*	18.8
Gastrointestinal symptoms	5	6.8
Continuing therapy	13	13.7
Total	102	22.6

* Case *R.G.* (*see* table 2) included

RESULTS

A summary of therapy with pyrazinamide in the study group is shown in table 1. The planned therapy was completed in 26 courses treated for an average of 35.3 weeks. Therapy was interrupted by the patient, by transfer, or by premature discharge in 37 courses after an average of 20.6 weeks of therapy. Two patients died of

far advanced tuberculosis during therapy with pyrazinamide. Their deaths did not appear in any way to be related to the drug.

Pyrazinamide was discontinued because of gastrointestinal symptoms in 5 patients and because of serum enzyme elevations in 18 patients. Therapy was stopped in an additional patient (*R.G.*) because of anorexia and the development of an enlarged tender liver, although the only laboratory evidence of hepatic dysfunction was a borderline elevation of the transaminase value. Significant transaminase elevations did not appear until after the drug had been discontinued. This patient has made an uneventful recovery. Therapy was not interrupted in any patient solely because of increased bromsulphalein retention or because of the development of an abnormality of any of the other conventional liver function tests. A detailed description of the cases in which significant enzyme elevations appeared is presented in table 2.

It was impossible to apply uniform criteria for interrupting therapy in all patients receiving pyrazinamide. The indications for the use of pyrazinamide ranged from an attempt to cover the critical period of resectional surgery performed in patients whose sputum cultures were positive with drug-resistant tubercle bacilli to a randomized research protocol which included pyrazinamide for patients with uncomplicated disease. The final decision for discontinuing the drug rested with the patient's ward physician; however, the recommendations of one of the investigators (J.F.M.) were followed in most cases. In general, there was more reluctance to withhold the drug early during a planned course of therapy or when significant therapeutic effect appeared to be taking place.

From an experimental standpoint, it would have been desirable to have continued therapy with pyrazinamide in the presence of serum enzyme elevations until a time when one or more of the conventional liver function tests showed significant abnormality. In view of the well-demonstrated relationship between cellular necrosis and serum transaminase elevations, and in view of the rapidity with which fatal pyrazinamide hepatitis had developed in the past (8), it was not believed to be justified to continue therapy when enzyme levels were significantly elevated. As indicated in table 2, it is not believed that all of the patients who developed significant enzyme elevations had pyrazinamide hepatitis or necessarily had liver damage due to any other cause. Four patients were receiving para-aminosalicylic acid (PAS), which will occasionally cause transaminase elevations. This drug may have played a roll in at least 2 of the reactions observed. Five of these reactions occurred in very ill patients with long-standing far advanced disease. Two of these patients died of their disease several months following complete recovery from their pyrazinamide reactions. One episode of enzyme elevation appeared to be due to a period of heavy ingestion of alcohol by a patient while he was on leave from the hospital. Thirteen of the reactions were considered either definitely or probably due to pyrazinamide.

Of the conventional liver function tests used to follow these patients, only the bromsulphalein was of value in confirming the presence of hepatic toxicity. Of the 11 reactions probably due to pyrazinamide, when concurrent bromsulphalein determinations had been performed, 7 were elevated but only 2 were above 10 per cent retention. In only one of these cases did the rise in bromsulphalein retention coincide with the rise in serum enzyme levels. Nonspecific transient bromsulphalein elevations were encountered as frequently in the study as nonspecific transaminase elevations. Several patients developed hypersensitivity reactions to the dye which prevented further screening with the agent. An additional group of patients had such poor veins that the test was omitted to prevent possible painful subcutaneous extravasations of the dye. Alkaline phosphatase determinations, cephalin flocculation, and thymol turbidity tests remained normal in almost all instances. The highest total serum bilirubin encountered in the study was 0.8 mg. per 100 ml. The prothrombin time was abnormal in several of the very sick patients; but changes could not be correlated with pyrazinamide therapy.

In figure 1, the enzyme changes which occurred during therapy and following test doses of pyrazinamide are graphically presented for the 3 patients in the group who have been shown most definitely to have developed a hypersensitivity to the drug. For simplicity, only the curves for the serum glutamic oxalacetic transaminase have been shown. The data for the pyruvic transaminase in these patients are incomplete but, as may be seen in table 2, tended to parallel those for the oxalacetic transaminase. Patient *L.M.* developed severe anorexia, malaise, and abdominal

TABLE 2

DETAILED SUMMARY OF ALL OF THE COURSES WHICH WERE INTERRUPTED BECAUSE OF SERUM TRANSAMINASE ELEVATIONS

Patient	Extent of Tuberculosis	Reason for Therapy	Drugs Used with Pyrazinamide	Weeks of Therapy	Enzyme Level when Pyrazinamide Was Stopped		Borderline Elevations Present before Reaction	Maximal Level Reached during the Reaction		Maximal Rise after a Test Dose of Pyrazinamide		Peak Bromsulphalein Retention during the Period of Enzyme Elevation	Diagnostic Impression of the Likelihood of Hepatic Toxicity Due to Pyrazinamide	Comment
					Serum Glutamic Oxalacetic Transaminase	Serum Glutamic Pyruvic Transaminase		Serum Glutamic Oxalacetic Transaminase	Serum Glutamic Pyruvic Transaminase	Serum Glutamic Oxalacetic Transaminase	Serum Glutamic Pyruvic Transaminase			
												per cent		
H.L.	F.A.	Chr.	SM, INH	45	47	36	+	81	215	—	—	6.5	Probable	Reaction occurred while patient was receiving prednisolone, 10 mg. daily.
H.F., first course	F.A.	Chr.	SM, INH	9	50	38	+	50	38	24	18	18.0	Probable	No hypersensitivity demonstrated.
H.F., second course	F.A.	Chr.	PAS, INH	44	152	187	−	152	187	—	—	—	Probable	Patient's condition was very poor, with severe hemoptysis.
J.R.	F.A.	Surg.	PAS, INH, VM	6	38	20	+	46	35	76	46	5.5	Definite	Reaction occurred in immediate postoperative period; pretreatment bromsulphalein 12%.
L.M.*	M.A.	Chr.	INH	10	176	—	−	176	—	162	91	9.5	Definite	Hypersensitivity demonstrated 14 weeks after the reaction.
J.S.*	M.A.	Surg	SM, INH	32	82	—	+	82	—	—	—	1.0	Probable	Elevation occurred coincident with the planned termination of therapy.
J.K.*	F.A.	Prot.	INH	17	43	68	+	43	68	—	—	2.0	Probable	Elevations persisted for three months.
K.R.*	F.A.	Chr.	INH	7	52	—	+	78	—	228	290	5.5	Definite	Attempts to desensitize this patient to pyrazinamide failed.
J.F.K.	F.A.	Chr.	INH, VM	21	38	9	+	38	18					

J.K.*	F.A.	Prot.	INH	7	52	—	+	78	—	228	200	5.5	Definite	pyrazinamide failed.
K.R.*	F.A.	Chr.	INH											
J.F.K.	F.A.	Chr.	INH, VM	21	38	9	+	38	18	—	—	2.0	Doubtful	Terminal patient who died after return of enzymes to normal.
L.H.	M.A.	Prot.	INH	16	30	40	+	65	45	—	—	3.0	Probable	Elevations persisted for three months; patient also sensitive to PAS.
F.L.	F.A.	Chr.	VM, INH, PAS	66	80	37	+	80	37	—	—	0.5	Possible	PAS could also be implicated here although enzyme level fell when pyrazinamide was discontinued.
A.S.	F.A.	Surg.	INH, SM	34	16	30	+	19	30	20	14	7.5	Unlikely	A second course of pyrazinamide well tolerated.
L.G.	F.A.	Chr.	—	42	59	60	+	59	60	42	15	20.0	Probable	Terminal patient who died after recovery from reaction.
R.G.*	M.A.	Prot.	INH	4	6	21	+	56	75	—	—	7.5	Probable	Liver enlarged and tender when drug was stopped.
E.P.	F.A.	Prot.	INH	21	56	54	+	76	65	—	—	6.5	Probable	
J.A.	F.A.	Chr.	INH, CYC	4	39	7	+	59	15	53	8	—	Doubtful	Patient runs erratic high serum glutamic oxalacetic transaminase with normal serum glutamic pyruvic transaminase; second course well tolerated.
F.O.	F.A.	Chr.	VM, INH	5	103	147	+	103	147	23	12	—	Probable	Patient noted anorexia, nausea on pyrazinamide; second course well tolerated.
R.P., first course	F.A.	Surg.	SM, PAS, INH	4	55	30	+	55	30	25	17	—	Unlikely	Reaction occurred one week postoperatively in company with reactions to penicillin and PAS.
R.P., second course	F.A.	Surg.	SM, INH	11	46	26	—	46	26	19	11	—	Unlikely	Enzyme elevation occurred after heavy drinking; third course tolerated well.

* Patients who underwent liver biopsy.

F.A.—far advanced; M.A.—moderately advanced; Chr.—chronic; Surg.—surgical; Prot.—protocol; SM—streptomycin; INH—isoniazid; PAS—para-aminosalicylic acid; VM—viomycin; CYC—cycloserine.

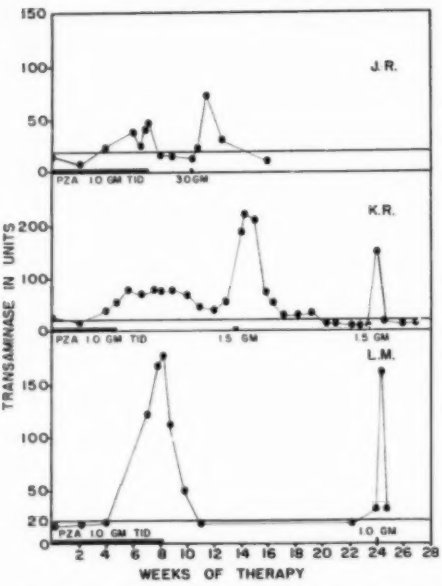

FIG. 1. Serum enzyme changes following test doses of pyrazinamide in 3 patients who developed hypersensitivity to the drug.

pain coincident with serum enzyme elevations after a test dose of 0.5 gm. of pyrazinamide. Because of reactions such as this and because of more severe reactions manifested by high fever, signs of meningeal irritation, skin eruptions, and abnormalities of liver function seen in PAS-susceptible patients when challenged with the latter drug, no attempt was made to challenge all of the patients who reacted to pyrazinamide. The administration of test doses was restricted to those patients for whom no other equally effective antituberculous agent was available.

Patient *K.R.* had far advanced cavitary pulmonary tuberculosis which had failed to respond to long courses of all of the other available antituberculous agents. He had shown some roentgenographic improvement while receiving pyrazinamide and conceivably could have benefited from further therapy with this drug. As may be seen in figure 1, he developed a rather marked hypersensitivity to the drug. Attempts to desensitize him were not completely successful and, unfortunately, were interrupted by his premature discharge. Of the remaining 16 patients in whom pyrazinamide therapy was discontinued because of serum enzyme elevations, 5 eventually completed their planned pyrazinamide treatment; 2 needed additional therapy (*L.M.* and *J.R.*) but became well and left the hospital without it; 4 were patients treated on a set protocol who have been transferred to other equally effective chemotherapy; and 5 who had chronic disease had received the drug from twenty-one to sixty-six weeks and had not benefited from it.

A summary of all of the enzyme determinations performed before treatment and during therapy with pyrazinamide is shown in table 3. The distribution of values before and during therapy was essentially the same. There was no great difference between the two enzyme tests. As may be seen, at any time nearly 80 per cent of the values would be within the normal range, approximately 15 per cent would be slightly elevated, and only 5 per cent of the values would be high enough to raise a question of pyrazinamide hepatitis.

The frequency of elevated pretreatment values noted in this study can be explained by the large number of patients with very far advanced disease in the group, the large number of alcoholics with variable degrees of hepatic cirrhosis, and the frequency with which their prior chemotherapy had included PAS. Other investigators, for example Allison (22), excluded all patients with abnormal liver function tests or a history of hepatitis, cirrhosis, or alcoholism from pyrazinamide treatment in the belief that such patients were more likely to suffer pyrazinamide reactions.

TABLE 3

SUMMARY OF ALL TRANSAMINASE VALUES PERFORMED BEFORE AND DURING PYRAZINAMIDE THERAPY

Determination	Number	Per Cent
Serum glutamic oxalacetic transaminase: in units		
Normal: 0–20	918	78.19
Borderline: 20–30	204	17.38
Elevated: 30+	52	4.43
Total	1,174	100.00
Serum glutamic pyruvic transaminase: in units		
Normal: 0–16	551	79.74
Borderline: 17–26	94	13.60
Elevated: 27+	46	6.66
Total	691	100.00

TABLE 4

SERUM LACTIC DEHYDROGENASE LEVELS ON SERUMS WITH SIGNIFICANT ELEVATIONS OF BOTH SERUM GLUTAMIC OXALACETIC TRANSAMINASE* AND SERUM GLUTAMIC PYRUVIC TRANSAMINASE† DUE TO PYRAZINAMIDE TOXICITY

Serum Lactic Dehydrogenase Levels	Number	Per Cent
Normal: 28–72 units	35	87.5
Equivocal: 73–90 units	0	0.0
High: >90 units	5	12.5
Total	40	100.0

* >30 units
† >26 units

TABLE 5

SERUM MALIC DEHYDROGENASE LEVELS ON SERUMS WITH SIGNIFICANT ELEVATIONS OF BOTH SERUM GLUTAMIC OXALACETIC* AND PYRUVIC† TRANSAMINASE DUE TO PYRAZINAMIDE TOXICITY

Serum Malic Dehydrogenase Levels	Number	Per Cent
Normal: 6–10 units	10	34.5
Equivocal: 11–15 units	13	44.8
High: >15 units	6	20.7
Total	29	100.0

* >30 units
† >26 units

Of the present study group of 95 patients, 38 would have been excluded by rigid application of the above criteria. It is of interest that 15.8 per cent of the group with a history of alcoholism or liver disease developed enzyme elevations on pyrazinamide, compared with 19.3 per cent of the group with negative histories in these two particulars and normal pretreatment function studies.

The experience with lactic dehydrogenase is shown in table 4. These 40 determinations were made on sera obtained during the study when pyrazinamide toxicity was present and both the serum glutamic oxalacetic and pyruvic transaminases were significantly elevated. Only 5 of these serum lactic dehydrogenase determinations were in the definitely abnormal range, indicating that this enzyme determination is of little or no value in this situation. Elevations of the lactic dehydrogenase were usually nonspecific and bore little or no relation to obvious reactions to pyrazinamide.

A similar evaluation of the malic dehydrogenase is shown in table 5. The correlation with pyrazinamide toxicity is better than in the case of the lactic dehydrogenase; 20.7 per cent of the values for malic dehydrogenase were significantly elevated and 44.8 per cent were in the equivocal range. The elevations of malic dehydrogenase tend to be smaller in magnitude than those of the glutamic oxalacetic or pyruvic transaminase levels. However, there is no question that the enzyme was elevated in most of the pyrazinamide reactions. The high concentration of malic dehydrogenase in erythrocytes makes its assay difficult to apply to routine clinical use because great care must be taken in drawing and handling blood samples to avoid significant hemolysis.

Five patients underwent needle biopsy of the liver during pyrazinamide reactions. None of these biopsies showed significant necrosis of parenchymal cells such as had been observed in the reported fatal cases of pyrazinamide hepatitis (6, 8). There were minor variations in the size of hepatic cells and their nuclei. Hepatic parenchymal cell necrosis as manifested by satellitosis was prominent in 2 cases and present in all. In one case these areas of necrosis were infiltrated with polymorphonuclear leukocytes. Four cases showed infiltration of portal triads with inflammatory cells, mostly lymphocytes. Hepatic parenchymal cells showed minor pigment deposition in 3 cases.

DISCUSSION

In all large series of published cases, jaundice has been regularly encountered in patients treated for the length of time and with the dose of pyrazinamide used in the present study, in which the patients have been followed by serial determination of one or more of the conventional liver function tests. It is assumed that, if the enzyme determinations had not been made, some of the patients would have become jaundiced before serial determinations of the bromsulphalein or of the other conventional tests would have warned of impending serious toxicity. Because of this failure of the conventional tests to protect patients from serious toxicity in other studies, it did not appear justified to continue therapy when significant elevations of oxalacetic or pyruvic

transaminase occurred in the patients in the present study.

Wingo, Fairly, and Roye (17) described their experience with transaminase determinations in 1957 in a group of fifty patients treated with pyrazinamide. They encountered nine bromsulphalein elevations not associated with other evidence of toxicity, and continued the drug without incident. Six patients developed enzyme elevations, and the drug was stopped. These patients all had bromsulphalein elevations at the time pyrazinamide was discontinued. However, in the two cases which are described in full, the drug was continued for several weeks after minor transaminase elevations had occurred. Wingo and associates concluded that the oxalacetic transaminase was of value in the early detection of liver damage.

Phillips (18) compared the oxalacetic transaminase with a number of other liver function tests in nine patients under pyrazinamide treatment. He concluded that bromsulphalein and oxalacetic transaminase were the most sensitive tests. Of the nine patients studied, five developed transaminase elevations. Four of these patients were continued on the drug and did not become jaundiced. It was concluded that the test was too sensitive for use as a clinical guide. Whether these enzyme elevations were persistent or simply isolated nonspecific elevations is not stated.

Wroblewski and LaDue selected 40 units as the upper limit of normal values for the transaminases. Wingo, Fairly, and Roye and most of the other investigators who have studied the oxalacetic transaminase have used this figure as the upper limit of normal. The present studies have shown that the clinical value of oxalacetic and pyruvic transaminase determinations in pyrazinamide-treated patients is increased if a narrower normal range based on the statistical distribution of transaminase levels in a normal population is used rather than one with an arbitrarily selected upper limit of normal. Serial studies in this series of patients have shown "warning" elevations of transaminase in 16 of the 19 reactions which were below this arbitrarily designated upper limit of normal for several days to several weeks before obvious toxicity appeared. In only one patient (*L.M.*) in whom pyrazinamide toxicity was likely was the initial elevated transaminase value above 40 units. Allison (22) has recently emphasized the importance of stopping the drug when minor abnormalities of the conventional liver function tests appear. Using this principle, he was able to reduce but not eliminate jaundice from his series. He used three liver function tests and studied the patients at fortnightly intervals. The present writers believe that either of the transaminase determinations will perform this same function with greater efficiency. When slight elevations appear, the frequency of the enzyme determinations can be increased. Elevations, particularly in the borderline range, may be transient and without obvious explanation. They are seen in association with hemoptyses, intercurrent respiratory infections, influenza-like illnesses, and reactions to other drugs such as penicillin, viomycin, or PAS. Elevations will occur in the first two weeks following major surgery in all patients and, when complications arise or when the surgery is quite extensive (such as pneumonectomy), elevations may occur later in the postoperative course. One patient in the present study developed a marked elevation of transaminase while receiving high doses of sodium salicylate. The salicylate was stopped and the enzyme level returned rapidly to normal while pyrazinamide therapy was continued. The elevation of transaminase during salicylate therapy was originally observed in children with rheumatic fever by Manso, Taranta, and Nydick (23). Patients have been observed to have transient elevations of enzyme following a period of heavy alcohol intake during leaves of absence from the hospital. The interpretation of an elevation of transaminase in a patient receiving narcotics should be made cautiously. If, in addition, the patient has previously undergone cholecystectomy, elevations following narcotics would almost certainly be unrelated to pyrazinamide. This phenomenon was originally observed by Foulk and Fleisher (24), and similar observations have been made at this hospital.

When unexplained significant enzyme elevations occur (usually above 40 units) and pyrazinamide toxicity appears likely, the drug should be discontinued. When the enzyme levels return to normal and further therapy is indicated, a test dose of the drug is administered. If enzyme elevations occur within twenty-four to seventy-two hours, as they did in 4 of the 10 patients tested, it is interpreted as indicating that the patient has developed specific drug hypersensitivity. Further pyrazinamide therapy should not be

attempted in such persons. It is believed that the risk of a test dose should not be taken unless further pyrazinamide therapy is necessary for the management of the patient's disease. Transaminase determinations are effective guides to drug dosage if desensitization is to be attempted, but desensitization per se is hazardous. It is believed that the 18 per cent of the patients who developed significant elevations of transaminase during pyrazinamide therapy, particularly those who were shown later to have drug hypersensitivity, included the 3 to 4 per cent of patients who would have developed jaundice if the drug had been continued longer. It is believed that it is preferable to interrupt, usually briefly, the pyrazinamide therapy of 18 per cent of the patient group in order to avoid more serious reactions in 3 to 4 per cent of this group.

Very little difference was found between the serum glutamic oxalacetic and the pyruvic transaminase determinations in the detection of pyrazinamide toxicity. Either assay is satisfactory for the purpose. The methods for assaying the enzymes are of equal complexity. There may be a slight increase in sensitivity if both enzymes are measured; but it is not believed that this additional safeguard is necessary for the routine case.

If decisions about therapy are to be based on relatively minor changes in the level of serum transaminase, it is essential that the determination be performed with great care using a sensitive method such as that of Karmen, Wroblewski, and LaDue. The care required in the collection and handling of blood to prevent hemolysis and to avoid deterioration cannot be overemphasized.

A comparison of the record of giving 102 courses of pyrazinamide without encountering jaundice with the 4.4 per cent incidence of jaundice encountered in the 801 cases reviewed from the literature by the chi-square method, utilizing Yates' correction factor, gives a square of 3.67, which is just slightly lower than the 3.84 value needed for significance at the 0.05 level. Utilizing a different statistical approach, that of sequential analysis, as described originally by Wald (25) and more recently by Doering and associates (26) and Hagans and associates (27), it can be shown that the serum transaminase should not be rejected in favor of conventional liver function tests at the 0.02 level of significance when 95 patients have been treated successfully without encountering jaundice.

Summary

Hepatic toxicity is a frequent complication of pyrazinamide therapy. The serum glutamic oxalacetic and the serum glutamic pyruvic transaminase determinations are very sensitive tests to detect its presence. Significant enzyme elevations suggesting liver injury appeared in 19 of the 102 courses of pyrazinamide administered to 95 patients. Four of these patients were later shown to have developed a specific hypersensitivity to pyrazinamide. It is believed that the pyrazinamide-treated patient followed by determination of either the serum glutamic oxalacetic or the serum glutamic pyruvic transaminase at two-week intervals receives optimal protection from serious liver damage insofar as can be provided by presently available liver function tests. The serial use of transaminase determinations will help prevent serious and fatal instances of drug toxicity. Either the serum glutamic oxalacetic transaminase or the serum glutamic pyruvic transaminase determinations can be used. The serum transaminase has been found to be a simple, economical, reliable laboratory test which can be performed in most clinical laboratories.

Sumario

El Descubrimiento de Lesiones Hepáticas Inducidas por la Piracinamida por medio de Determinaciones de Enzimas en el Suero Sanguíneo

La intoxicación hepática constituye una complicación común de la piracinamidoterapia. Las determinaciones séricas de las transaminasas glutámica oxalacética y glutámica pirúvica son pruebas muy delicadas para distinguir su presencia. Importantes elevaciones enzimáticas que indicaban lesión hepática aparecieron en 19 de las 102 series de piracinamida administradas a 95 enfermos. En 4 de estos enfermos se descubrió después que habían adquirido hipersensibilidad específica a la piracinamida. Parece que el enfermo tratado con piracinamida al cual se le ejecutan a plazos de dos semanas determinaciones ya de la serotransaminasa glutámica oxalacética o la serotransaminasa glutámica pirúvica recibe protección óptima contra las lesiones hepáticas graves hasta donde permiten hacerlo las pruebas de la función hepática disponibles actualmente. El empleo seriado de las determinaciones de las transaminasas ayudará a impedir los casos graves y letales de intoxicación medicamentosa. La

determinación de la trasaminasa sérica ha resultado ser una prueba sencilla, económica y fidedigna de laboratorio, que puede ejecutarse en la mayoría de los laboratorios clínicos.

Resume

Détection des troubles hépatiques causés par la pyrazinamide par dosage de l'enzyme sérique

L'intoxication hépatique est une complication fréquente du traitement par la pyrazinamide. Les dosages de la transaminase glutamique oxalo-acétique et glutamique pyruvique du sérum sont des tests très sensibles, permettant de déceler son existence. Des élévations marquées du taux enzymatique indiquant une lésion hépatique ont été constatées au cours de 19 sur 102 traitements par la pyrazinamide administrée à 95 malades. Quatre de ces malades ont par la suite présenté une hypersensibilité spécifique à la pyrazinamide. Les auteurs estiment que dans l'état actuel des tests, pour éviter des lésions graves du foie, il convient d'effectuer régulièrement, toutes les deux semaines, un dosage soit de la transaminase glutamique oxalo-acétique du sérum, soit de la transaminase glutamique pyruvique du sérum, chez les malades traités par la pyrazinamide. Ces dosages systématiques de la transaminase aideront à prévenir l'apparition de cas d'intoxication médicamenteuse grave et peut-être fatale. Il a été constaté que la transaminase sérique est un test de laboratoire simple, économique et sûr, qui peut être effectué dans la plupart des laboratoires cliniques.

Acknowledgments

The writers would like to express their appreciation to the following members of the staff of the Madison Veterans Administration Hospital who assisted them in this project: Dr. D. S. Stevenson, who reviewed all of the liver biopsy sections; Doctors A. B. Falcone and D. S. Goldman for their help in setting up the assays for the serum lactic and malic dehydrogenases; and Dr. M. M. Stone for her management of the attempted desensitizations to pyrazinamide and her general support and interest in the study. The writers would also like to thank Dr. D. A. Grant of the University of Wisconsin, Department of Psychology, for evaluating their statistical analysis.

REFERENCES

(1) Yeager, R. L., Monroe, W. G. C., and Dessau, F. I.: Pyrazinamide (Aldinamide) in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1952, *65*, 523.

(2) Campagna, M., Calix, A. A., and Hauser, G.: Observations on the combined use of pyrazinamide (Aldinamide) and isoniazid in the treatment of pulmonary tuberculosis: A clinical study, Am. Rev. Tuberc., 1954, *69*, 334.

(3) Allison, S. T.: Pyrazinamide-isoniazid in low dosage in treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *74*, 400.

(4) Phillips, S., Larkin, J. C., Jr., Litzenberger, W. L., Horton, G. E., and Haimsohn, J. S.: Observations on pyrazinamide (Aldinamide) in pulmonary tuberculosis, Am. Rev. Tuberc., 1954, *69*, 443.

(5) Phillips, S., and Horton, G. E.: Pyrazinamide-isoniazid: Comparison with isoniazid–para-aminosalicylic acid in active pulmonary tuberculosis with the choice of regimens determined by chance, Am. Rev. Tuberc., 1956, *73*, 704.

(6) Potter, B. P., and Chang, S. F.: Experience with pyrazinamide, Dis. Chest, 1955, *27*, 44.

(7) Ewart, G. E., and Roye, W. E.: Pyrazinamide-isoniazid in the treatment of pulmonary tuberculosis, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 78.

(8) McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R. M., Jr., and Tompsett, R.: Pyrazinamide-isoniazid in tuberculosis, Am. Rev. Tuberc., 1954, *69*, 319.

(9) Muschenheim, C., Organick, A., McCune, R. M., Jr., Batten, J., Deuschle, K., Tompsett, R., and McDermott, W.: Pyrazinamide–isoniazid in tuberculosis: III. Observations with reduced dosage of pyrazinamide, Am. Rev. Tuberc., 1955, *72*, 851.

(10) Benson, W. P., and McLean, R. L.: PZA combined with viomycin in treatment of far advanced pulmonary tuberculosis, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 79.

(11) Krasner, L.: PZA in ten cases of chronic pulmonary tuberculosis, Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army and Navy, 1953, p. 341.

(12) Schwartz, W. S.: Comment, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 110.

(13) McDermott, W.: Comment, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 82.

(14) Committee on Therapy of the American Trudeau Society: A statement on pyrazinamide, Am. Rev. Tuberc., 1957, *75*, 1012.

(15) Wroblewski, F., and LaDue, J. S.: Serum glutamic oxalacetic aminopherase (transaminase) in hepatitis, J.A.M.A., 1956, *160*, 1130.

(16) Morrissey, J. F.: The early detection of

pyrazinamide hepatitis by means of the serum transaminase, Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces, 1957, p. 264.

(17) Wingo, C. F., Fairly, J. L., and Roye, W. E.: The use of the serum glutamic oxalacetic transaminase as a screening test for liver toxicity in patients receiving pyrazinamide for tuberculosis, Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces, 1957, p. 254.

(18) Phillips, S.: Tests for PZA-induced hepatotoxicity, Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration–Armed Forces, 1957, p. 262.

(19) Karmen, A., Wroblewski, F., and LaDue, J. S.: Transaminase activity in human blood, J. Clin. Invest., 1955, *34*, 126.

(20) Karmen, A.: A note on the spectrophotometric assay of glutamic oxalacetic transaminase in human blood serum, J. Clin. Invest., 1955, *34*, 131.

(21) Neilands, J. B.: Lactic dehydrogenase of heart muscle, in *Methods in Enzymology*, S. D. Colowick and N. O. Kaplan, ed., Academic Press, New York, 1955, Vol. I, p. 449.

(22) Allison, S. T.: Pyrazinamide in low dosage in combination with isoniazid or para-aminosalicylic acid in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1959, *79*, 102.

(23) Manso, C., Taranta, A., and Nydick, L.: Effect of aspirin administration on serum glutamic oxalacetic and glutamic pyruvic transaminases in children, Proc. Soc. Exper. Biol. & Med., 1956, *93*, 84.

(24) Foulk, W. T., and Fleisher, G. A.: The effect of opiates on the activity of serum transaminase, Proc. Staff Meet., Mayo Clin., 1957, *32*, 405.

(25) Wald, A.: Sequential Analysis, John Wiley and Sons, Inc., New York, 1947, p. 88.

(26) Doering, C. R., Hagans, J. A., Ashley, F. W., Clark, M. L., and Wolf, S.: Sequential analysis in therapeutic research: Application to binomial data and to measured data normally distributed (one-sided alternative), J. Lab. & Clin. Med., 1957, *50*, 621.

(27) Hagans, J. A., Doering, C. R., Clark, M. L., Schneider, E. M., and Wolf, S.: Sequential analysis in therapeutic research: II. Application to measured data normally distributed (two-sided alternative), J. Lab. & Clin. Med., 1957, *50*, 629.

DESOXYRIBONUCLEIC ACID AS GROWTH STIMULANT OF TUBERCLE BACILLI[1,2]

E. DARZINS

(Received for publication January 23, 1959)

Introduction

Many searches have been carried on for substances that will enhance the growth or multiplication of tubercle bacilli. As early as 1887 it was found that glycerol stimulates highly the growth of tubercle bacilli (1). It has also been observed that inorganic iron salts (2) and oleic acid (3) increase the speed of growth of tubercle bacilli. These nutrients must be metabolized by the microbial cell before they can influence the process of growth; they must be added in considerable amounts to the culture medium, and their action is slow.

The nuclear bodies of *M. tuberculosis* divide before the cell divides (4–6), and the nuclear substance controls the division of a cell. The content of nuclear substance of a cell is doubled before onset of nuclear division (7, 8). A nuclear substance may be able to enter a cell and act upon the growth or multiplication-regulating mechanism of the nucleus. The guiding idea of the work reported here was to obtain, if possible, a pure nuclear substance of tubercle bacilli and investigate its action on the speed of growth of tubercle bacilli.

Material and Methods

Pathogenic human H37Rv and bovine Ravenel strains of tubercle bacilli were grown for three weeks on 50 ml. of liquid medium[3] in 250-ml. Erlenmeyer flasks. Bacilli were collected in 250-ml. centrifuge tubes and washed six times with 200 ml. of 0.02 M sodium citrate solution until they were free from pigment. After washing, 77 gm. of bacilli (wet weight) remained. The washed bacilli were transferred into two 500-ml. Pyrex bottles with glass stoppers. Each bottle also contained 200 glass beads 3 mm. in diameter; 15 gm. of glass powder (Fisher G-17); and 250 ml. of a solution composed of 0.02 M sodium citrate, 0.1 M sodium chloride, and 0.08 M borate buffer—total M 0.2, pH 8.1. These bottles were fastened in a box in a horizontal position and the box was put on a Kahn shaker making two hundred seventy-five 3-cm. strokes per minute. The direction of shaking was parallel to the lengthwise axis of the bottles. The bottles were shaken for a total of twenty-four hours, six hours daily. During the rest of the time the box containing the bottles was kept in a refrigerator. After the shaking was completed, the bacillary suspension was centrifuged for one hour at 2,000 r.p.m. The supernatant was decanted and poured into chilled 95 per cent ethanol (two volumes of alcohol per volume of supernatant). Long, fine threads of white substance appeared. This liquid was immediately centrifuged, and the deposit was treated with 0.1 M sodium chloride solution. After two more alcohol precipitations, the solution of the substance was transferred into a separatory funnel and 0.2 volume of chloroform and 0.1 volume of *n*-amyl alcohol were added to each volume of solution; then the mixture was vigorously shaken for five minutes. The emulsion that formed was centrifuged and the supernatant precipitated with alcohol. After two more alcohol precipitations, the white fibrous substance was dissolved in sterile 0.001 M phosphate buffer of pH 7.0 and kept in a refrigerator. From 3.38 gm. of dry bacilli, 0.016 gm. of this substance was obtained (0.47 per cent).

All manipulations with live pathogenic bacilli were carried out in closed sterile systems of containers.

Observations

Identification of the Isolated Substance

The obtained substance showed strong Dische's reaction and negative biuret, Millon, and Salkowski reactions. Orcinol reaction was doubtful with this substance, and Sudan III and osmium staining was negative. This substance when dissolved in 0.001 M phosphate buffer of pH 7.0 and observed in a 1-cm. cell had its maximal ultraviolet light absorption at 258 mμ wavelength[4] (figure 1).

The substance contained 15 per cent nitrogen and 8.7 per cent phosphorus; the atomic ratio

[1] From the Research Laboratory, Anoka State Hospital, Anoka, Minnesota.

[2] Presented at the meeting of the Minnesota Trudeau Medical Society, Minneapolis, November 28, 1958.

[3] The composition of media used in this work is given in the writer's book, *The Bacteriology of Tuberculosis*, University of Minnesota Press, Minneapolis, 1958.

[4] This determination was made by Dr. S. Kirkwood, University of Minnesota, St. Paul Campus.

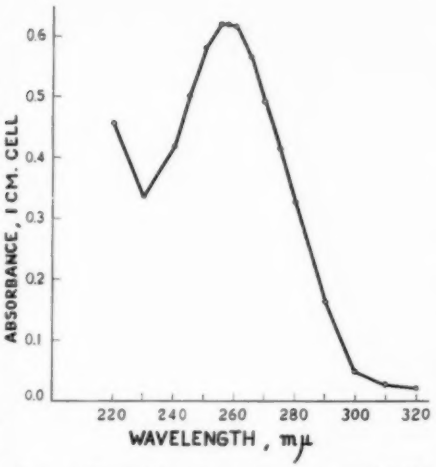

FIG. 1. Ultraviolet absorption spectra of DNA of tubercle bacilli in 0.01 M phosphate buffer at pH 7.0.

N/P was 3.81. Each milligram of this substance (dry weight) reduced an amount of copper sulfate which will be reduced by 1 ml. of 0.88 per cent glucose solution (Nelson's technique).

Solutions of the substance in saline containing 0.001 M magnesium sulfate were adjusted to pH 7.0. To each milliliter of these solutions, 100 γ of crystalline desoxyribonuclease[5] were added, and after incubation for two hours at 37°C., their optical densities were measured in Beckman's spectrophotometer at 259 mμ wave length. Steady decrease of densities, such as that from 0.740 to 0.490, was observed.[6]

The solutions treated with desoxyribonuclease were tested for their growth-stimulating action on BCG. Only slight stimulation, compared with that of the untreated controls, remained.

The mode of preparation and the physical and chemical properties of the obtained substance indicated that it was relatively pure desoxyribonucleic acid (DNA).

The material, when stained according to the Ziehl-Neelsen technique, was alcohol-acid fast. The solutions of this substance contained very few alcohol–acid-fast bacilli. The fine, long, and homogeneously stained threads of this substance cannot be confused with tubercle bacilli.

[5] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[6] This determination was made by Dr. M. O. Schultze, University of Minnesota, St. Paul Campus.

Standardization of the Obtained Desoxyribonucleic Acid

To compare the biologic activity of different samples of DNA, three standardization procedures were used:

(*1*) Solutions of DNA dried in a vacuum to constant weight and known weight of dry DNA were used in experiments; (*2*) to avoid the drying of DNA, it was standardized according to the intensity of Dische's reaction; 2 mg. per ml. of dry DNA produced a blue color in Dische's reaction which in an Evelyn photometer with filter 540 gave a reading of 50; by dissolving 4.1 mg. of toluidine blue (Harleco) in 1 liter of distilled water, an artificial standard was prepared which gave a reading of 50 on the photometer and remained stable for many months. This standard was used to check the color in Dische's reaction of unknown DNA solutions and to standardize their strength; (*3*) the biologic procedure was used when the constancy of DNA rates stimulating the growth of BCG was discovered.

Action of Desoxyribonucleic Acid on the Growth of BCG and Tubercle Bacilli

The generation time of tubercle bacilli in liquid medium is approximately sixteen to eighteen hours (9). The action of DNA on the formation of new progeny of BCG was most powerful in old, preincubated bacilli which grew poorly on solid media.

BCG culture was adapted to dispersed growth in basal medium containing 0.5 per cent Tween® 80. A tube of this medium was inoculated with a minute amount of three-month-old BCG culture which had been kept on malachite-egg medium in a refrigerator. After twenty hours of incubation at 37°C., a drop of this culture, containing approximately 430 viable bacillary units, was inoculated into a series of tubes, each containing 9 ml. of liquid medium and decreasing amounts of DNA. The first tube contained 200 γ of DNA per ml.; each succeeding tube had ten times less DNA than the preceding one. No DNA was added to the control tubes. All cultures were maintained at 37°C.

On the fifth day of incubation, the tube containing 2×10^{-4} γ per ml. of DNA showed a deposit on the bottom; after eight days of incubation the deposit became heavy. This deposit, when stained according to the Ziehl-Neelsen technique, revealed an abundance of cords of acid-fast bacilli. In this tube the growth was explosive

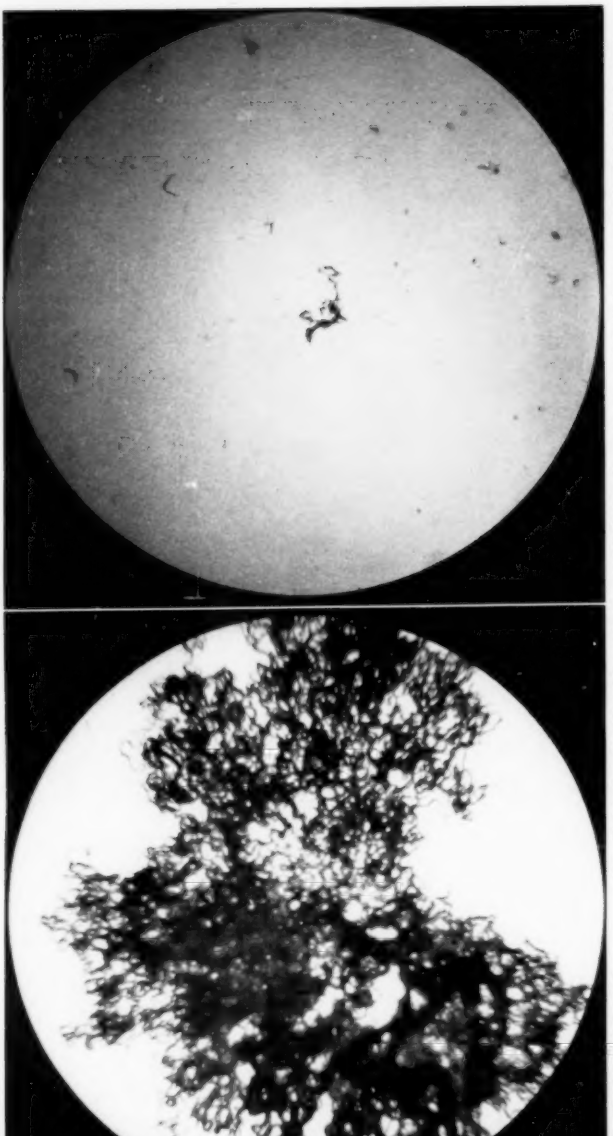

FIG. 2. (Left) BCG culture grown for eight days in liquid medium at 37°C. in the presence of 2×10^{-4} γ per ml. of DNA. (×100)
FIG. 3. (Right) BCG culture grown for eight days in liquid medium in the absence of DNA. (×100)

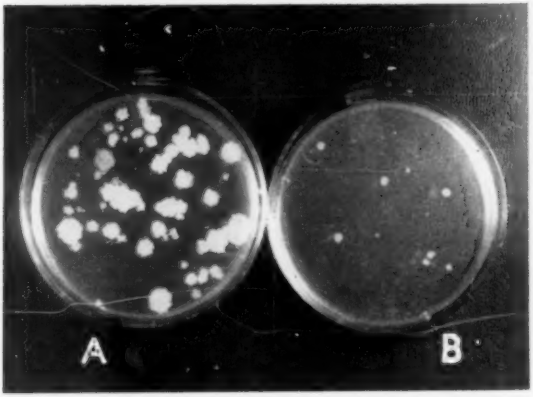

FIG. 4. BCG culture grown for fourteen days at 37°C. on serum-agar (reduced $\frac{2}{5}$). A — in the presence of 2×10^{-4} γ per ml. of DNA; B — in the absence of DNA.

compared with that in the controls: a network of cords covered the field of a microscope (figure 2), while only initial cords could be seen in the control tubes (figure 3). The mean diameter of the twenty largest colonies grown in the presence of DNA was 910 ± 33.6 μ; that of the colonies in the control tubes, 54 ± 1.23 μ. The deposit in the tube containing 2×10^{-4} γ per ml. of DNA and that in a control tube were collected, centrifuged, and dried. The mean dry weight of bacilli grown in the presence of DNA was 17 ± 2.4 mg.; that of controls, 4 ± 1.2 mg.

BCG cultures grown for fourteen days on serum-glycerol agar also showed enhanced growth compared with that in the controls (figure 4).

The growth acceleration by DNA was observed not only in BCG but also in the pathogenic H37Rv strain of tubercle bacilli. The DNA isolated from *Mycobacterium phlei* had only a feeble growth-enhancing effect on BCG.

The turbidimetric comparison in an Evelyn photometer of the speed of rate of growth of BCG cultures in the liquid medium containing Tween 80, with the added DNA and without it, showed that the difference in both cultures was the shortening of the lag period of the cultures growing in the presence of DNA.

The growth-promoting optimum of each DNA preparation showed remarkable constancy and was used for the biologic standardization of unknown DNA solutions.

DISCUSSION

The substance obtained from tubercle bacilli was precipitated with alcohol in the form of characteristic long white threads which showed strong Dische's reaction; its maximal ultraviolet light absorption was at 258 mμ; its atomic N/P ratio was 3.81. This substance had negative biuret and Millon reactions; fatty substances were absent; when treated with desoxyribonuclease this substance lost most of its growth-stimulating capacity. The sum of the physical, chemical, and biologic properties of this substance indicated that it was relatively pure desoxyribonucleic acid.

The explosive growth promoted by DNA in old populations of bacilli has had a maximum at 2×10^{-4} γ of DNA per ml. This amount of DNA is in the range of DNA of pneumococci needed to produce the transformation of apathogenic pneumococci into pathogenic ones (10) and initiating the intrabacterial growth of T2 bacteriophages (11).

The obtained DNA was alcohol-acid fast although fatty substances were absent from it.

The action of DNA derived from pathogenic tubercle bacilli on biologic properties and progeny of BCG is the subject of further study.

The observed effect of DNA on the growth of old, dying cultures of BCG and tubercle bacilli may be used in culturing the few altered bacilli from old tuberculous lesions, and may cast some light on the revival and spread of tubercle bacilli in tuberculous patients.

SUMMARY

Desoxyribonucleic acid (DNA) was obtained from living pathogenic tubercle bacilli. This substance was alcohol-acid fast. The solutions of

DNA were standardized by three different procedures. The explosive growth-promoting action of DNA was observed in old, degenerating cultures of BCG and tubercle bacilli kept for three months in a refrigerator. The obtained DNA has growth-promoting maximum at 2×10^{-4} γ per ml. In a liquid medium the growth-promoting effect of DNA can be seen in cultures incubated at 37°C. for eight days; on solid serum-agar medium it can be observed in fourteen days. Desoxyribonucleic acid isolated from *Mycobacterium phlei* had a feeble growth-promoting effect on BCG cultures. In the presence of DNA in a medium, the lag period of BCG is shortened and the speed of growth increased.

Sumario

El Acido Desoxirribonucleico como Excitador del Crecimiento de los Bacilos Tuberculosos

Se obtuvo ácido desoxirribonucleico (ADN) de bacilos tuberculosos patógenos vivos. Esta substancia era alcohol-ácido-resistente. Las soluciones de ADN fueron normalizadas por tres procedimientos distintos. La acción explosiva y fomentadora del desarrollo del ADN fué observada en cultivos viejos en vías de degeneración de BCG y en bacilos tuberculosos conservados por tres meses en el refrigerador. El ADN obtenido logra el máximo fomento del desarrollo a 2×10^{-4} γ por ml. En un medio líquido puede observarse el efecto fomentador del crecimiento del ADN an cultivos incubados a 37°C. durante ocho días; en el medio sólido de agar-suero puede observarse en catorce días. El ácido desoxirribonucleico aislado del *Mycobacterium phlei* mostró débil efecto de fomento del desarrollo en los cultivos de BCG. Cuando existe ADN en un medio, se acorta el período de espera del BCG y aumenta la velocidad de la proliferación.

Resume

L'acide désoxyribonucléique: un stimulant de la croissance des bacilles tuberculeux

L'acide désoxyribonucléique (DNA), qui est alcoolo-acido-résistant, a été extrait de bacilles tuberculeux pathogéniques. Les solutions de DNA ont été standardisées suivant trois procédés différents. L'effet stimulant puissant de DNA sur la croissance des bacilles a été observé sur de vieilles cultures dégénérées de BCG et de bacilles tuberculeux conservés pendant trois mois au congélateur. Le DNA ainsi obtenu avait un effet stimulant de croissance maximum de 2×10^{-4} γ par ml. En milieu liquide, l'effet stimulant de croissance du DNA peut être observé dans des cultures laissées à l'étuve à 37° C pendant 8 jours; en milieu solide sérum-agar, il peut s'observer au bout de 14 jours. L'acide désoxyribonucléique isolé du *Mycobacterium phlei* ne possède qu'un pouvoir stimulant de croissance faible sur les cultures de BCG. Par la présence de DNA dans le milieu, la période de retard du BCG est réduite et la vitesse de croissance augmentée.

REFERENCES

(1) Nocard and Roux: Sur la culture du bacille de la tuberculose, Ann. Inst. Pasteur, 1887, *1*, 19.

(2) Sauton, B.: Sur la nutrition minèrale du bacille tuberculeux, Compt. rend. Acad. Sc., 1912, *155*, 860.

(3) Dubos, R. J.: Effect of long-chain fatty acids on bacterial growth, Proc. Soc. Exper. Biol. & Med., 1946, *63*, 56.

(4) Knaysi, G.: The cytology and microchemistry of *Mycobacterium tuberculosis*, J. Infect. Dis., 1929, *45*, 13.

(5) Mudd, S., and Smith, A. G.: Electron and light microscopic studies of bacterial nuclei, J. Bact., 1950, *59*, 561.

(6) Ruska, H., Bringmann, G., Neckel, J., and Schuster, G. S.: Über die Entwicklung sowie den morphologischen und zytochemischen Aufbau v. *Mycobacterium avium* (Chester), Z. wiss. Mikr., 1952, *60*, 425.

(7) Alfert, M.: A cytochemical study of oogenesis and cleavage in the mouse, J. Cell. & Comp. Physiol., 1950, *36*, 381.

(8) Caspersson, T.: Über die Rolle der Desoxyribonukleinsäure bei der Zellteilung, Chromosoma, 1939/1940, *1*, 147.

(9) Youmans, G. P., and Youmans, A. S.: A method for the determination of the rate of growth of tubercle bacilli by the use of small inocula, J. Bact., 1949, *58*, 247.

(10) Avery, O. T., MacLeod, C. M., and McCarty, M.: Studies on the chemical nature of the substance inducing transformation of pneumococcal types: Induction of transformation by the desoxyribonucleic acid fraction isolated from pneumococcus Type III, J. Exper. Med., 1944, *79*, 137.

(11) Hershey, A. D., and Chase, M.: Independent function of viral protein and nucleic acid in growth of bacteriophage, J. Gen. Physiol., 1952, *36*, 39.

ANTITUBERCULOUS ACTIVITY OF A PHENAZINE DERIVATIVE (B663)[1]

B. K. ACHARYA, J. M. ROBSON, AND F. M. SULLIVAN

(Received for publication June 6, 1959)

INTRODUCTION

Barry and his co-workers have described the antituberculous activity of a number of phenazine derivatives; one of these (B663) is remarkably potent and also possesses striking cumulative properties, being stored in many organs and tissues for long periods after administration (1, 2). It seemed of interest to investigate the activity of this compound with the mouse corneal test, which has proved of value in determining the effects of a number of other antituberculous substances (3, 4).

EXPERIMENTAL

Two main types of experiments were performed. In the first, the organisms (*M. tuberculosis* var. *bovis*) were exposed to the drug *in vitro* and were subsequently inoculated into mice to determine their viability. In the second set of experiments, the mice were inoculated intracorneally and treated with the drug incorporated in their diet.

The experiments were carried out on albino mice of both sexes, weighing 20 to 25 gm. at the beginning of the experiment. The organism used was a bovine strain of *M. tuberculosis*, and the details of cultivation and intracorneal inoculation, as well as the method of scoring the developing lesions and measuring the number of bacilli, have previously been described (5). The method of treating the organisms *in vitro* will be described in the appropriate section. For administration to mice the drugs were incorporated in British Medical Research Council diet 41*B*, the daily dose per mouse being contained in 5 gm. of diet.

Intracorneal inoculation of organisms previously treated in vitro: To investigate the bactericidal effect of B663 *in vitro*, bacilli were recovered from fully grown cultures of *M. tuberculosis* var. *bovis* in Dubos medium. The organisms were then divided equally between three sets of tubes containing Proskauer and Beck medium enriched with 5 per cent of human serum. To one set of tubes, B663 dissolved in ethyl alcohol in a concentration of 40 γ per ml. was added to give a final concentration of 0.8 γ of B663 per ml., i.e., the concentration used by Barry and his co-workers (1); to another set of tubes the same amount of alcohol was added as was used to dissolve the B663; and the third set was left untreated. These tubes were then incubated at 37°C. After one week, two weeks, and three weeks of incubation, the cultures were diluted to 10^{-1} and 10^{-2} in 0.05 per cent Tween® saline, thoroughly shaken, and inoculated intracorneally into groups of 6 mice, which were then observed for a period of three to four months.

Results: All animals in all of the groups infected with the 10^{-1} dilution developed lesions after various intervals and, due to the relatively large inoculum (about 50,000 bacilli), marked differences were not seen. An obvious effect was seen, however, in the animals infected with the smaller inoculum. There was no significant effect after incubation for one week, but the cumulative number of animals which developed lesions in the groups inoculated with the organisms after two and three weeks' incubation is shown in figure 1. The addition of the alcohol alone to the medium did not produce any effect as compared with the untreated medium, so that, for clarity, only the results from this culture, i.e., medium plus alcohol, and the drug-treated culture are shown. It may be seen that the phenazine compound markedly reduced the number of animals which developed corneal lesions and also increased the latent period before lesions developed, as compared with those in the controls.

Thus, it is obvious that the compound B663 has some bactericidal effect when incubated with *M. tuberculosis in vitro* under the conditions described.

In order to determine whether the organisms take up this drug from the medium at the end of one week of incubation, a smear of the undiluted culture and a cornea inoculated with the same culture were examined under the microscope. In the smear examination, the coloration of the individual bacilli by the drug could not be definitely ascertained. When the cornea inoculated with the undiluted culture was examined, however, the individual organisms were found to be stained brown. Such stained organisms were seen quite clearly against the unstained background of the corneal tissue.

Effect of B663 alone in experimental corneal tuberculosis: Groups of 6 to 10 mice were inoculated intracorneally with either an undiluted or a 10^{-3}

[1] From the Department of Pharmacology, Guy's Hospital Medical School, London, England.

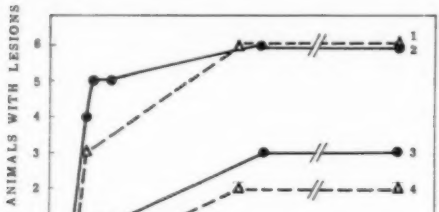

FIG. 1. Showing the number of mice developing corneal lesions after infection with a 10^{-3} dilution of *M. tuberculosis* var. *bovis*, previously incubated in a medium containing B663 for two and three weeks: (*1*) Incubated three weeks—medium plus alcohol; (*2*) incubated two weeks—medium plus alcohol; (*3*) incubated two weeks—medium plus B663; (*4*) incubated three weeks—medium plus B663.

dilution of a Dubos culture of virulent *M. tuberculosis* var. *bovis*. The group infected with an undiluted culture was given 1 mg. of B663 per mouse per day in the diet, and groups infected with a 10^{-3} dilution of the same culture were given either 100 or 50 γ of the drug per mouse per day in the diet. Groups of mice similarly inoculated with the two doses of bacilli received no treatment and were included as controls. Treatment was started on the day of inoculation and was continued for a period of twenty-eight days. These mice were observed for a period of twenty-one weeks.

Both at fourteen and at thirty-two days after the beginning of the experiment, a mouse of the group treated with 1 mg. of the drug daily was sacrificed, and the infected cornea was examined by phase-contrast microscopy and later by gross staining with dilute carbolfuchsin. The internal organs were examined for any accumulation of the drug.

Results: As may be seen in figure 2, B663 had a beneficial effect on the development of the infection which was evident not only during the period of treatment, but also after treatment was stopped. The average size of the lesions in the treated groups was smaller than in untreated controls. The effect was most marked in the group treated with the large dose of the drug. Complete suppression of the lesion was not seen in any animal of the group infected with an undiluted culture but, in the groups infected with the smaller number of organisms, an increased incubation period was seen in some and complete suppression of the lesions was observed in other animals.

In the animals killed for examination both at fourteen and at thirty-two days after infection, the findings were the same. At the time of removal, both corneas had lesions filling about one-eighth of the area. On examination by phase-contrast microscopy the lesions were found to be stained light brown to deep brown in places, and

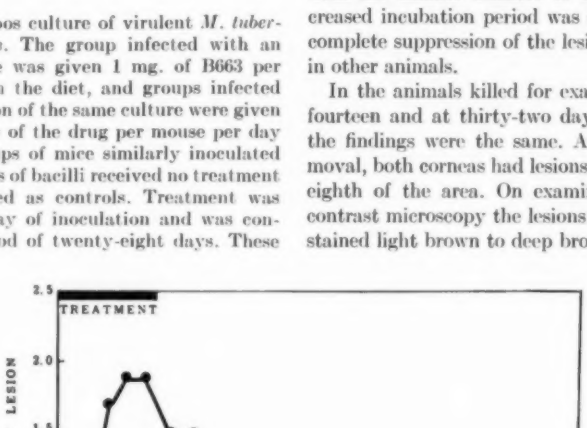

FIG. 2. Showing the effect of B663 on mice infected intracorneally with either an undiluted or a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis*: (*1*) infected with undiluted culture—control; (*2*) infected with 10^{-3} dilution—control; (*3*) infected with 10^{-3} dilution and treated with B663—100 γ; (*4*) infected with 10^{-3} dilution and treated with B663—50 γ; (*5*) infected with undiluted culture and treated with B663—1.0 mg. All doses expressed as amounts per mouse per day.

the macrophages at the periphery of the lesions were seen to contain brown granules of different sizes. This shows that the drug which is a brown dye penetrates into the lesion and also into the macrophages, and it is possible that it is even concentrated in the lesion. The bacilli could not be seen owing to the density of the lesion.

On gross staining with carbolfuchsin, the corneas showed large numbers of acid-fast bacilli which had multiplied to give dense aggregates with a *T* value greater than *T*7 over an area of 1 mm. in both instances.

On post-mortem examination of these animals, the subcutaneous and mesenteric fat, intestines, (especially large intestines), kidneys, and bladders were found to be stained yellow due to an accumulation of the dye in these tissues.

In conclusion, the phenazine derivative, B663, possesses an antituberculous activity which, although it does not suppress the formation of lesions in all animals, nevertheless results in the final lesion size being considerably smaller than that in the controls. Furthermore, effects are seen with doses as low as 50γ per mouse per day (equivalent to 2 mg. per kg.). The drug is accumulated in the animal tissues, particularly in fatty tissue, and also probably accumulates in the lesions.

Effect of B663 and isoniazid, alone and in combination, in experimental corneal tuberculosis: Twenty-eight mice were inoculated intracorneally with a 10^{-3} dilution of *M. tuberculosis* var. *bovis* and were divided into four groups: (*a*) control, untreated; (*b*) treated with 0.3 mg. of B663 per mouse per day in the diet; (*c*) treated with 0.3 mg. of isoniazid per mouse per day in the diet; and (*d*) treated with 0.3 mg. of B663 and 0.3 mg. of isoniazid per mouse per day in the diet. Treatment was started from the day of infection and was continued for a period of twenty-eight days, and the mice were observed for a period of sixteen weeks.

Results: The development of lesions in the various groups is shown in figure 3. A dose of 0.3 mg. of isoniazid per day completely suppressed the development of any macroscopic corneal lesion during the twenty-eight-day period of treatment but, after a further interval corresponding to the usual incubation period in untreated animals, lesions appeared, and in the following four weeks all of the mice of this group developed corneal lesions. On the other hand, in the group of mice treated with B663 alone, corneal lesions appeared during the period of

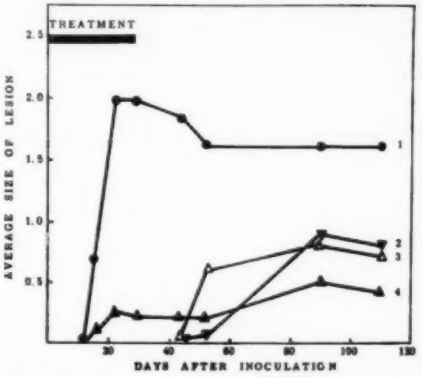

FIG. 3. Showing the effect of B663 alone and in combination with isoniazid in mice infected intracorneally with a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis*: (*1*) control—untreated; (*2*) B663—0.3 mg. and isoniazid—0.3 mg.; (*3*) isoniazid—0.3 mg.; (*4*) B663—0.3 mg. All doses expressed as amounts per mouse per day.

treatment, but the average size of the lesions at any time after the sixth week was smaller than that in the animals of the corresponding group treated with isoniazid alone. This suggests that, with the doses used, isoniazid was superior to B663 in suppressing the development of corneal lesions during the period of administration, whereas B663 had a more prolonged effect. When B663 and isoniazid were combined, there was complete suppression of the development of corneal lesions during the twenty-eight-day period of treatment but, after a further interval corresponding to the usual incubation period in the untreated animals, lesions appeared and the combination of B663 with isoniazid did not seem to produce any greater effect than when isoniazid was used alone.

Prophylactic effect of B663 in experimental corneal tuberculosis: The results of previous experiments have shown not only that this drug is beneficial in experimental tuberculosis, but also that it accumulates in the animal tissues. A further experiment was, therefore, instituted to study the prophylactic effect of this drug in experimental corneal tuberculosis.

A group of 12 mice were given 0.3 mg. of B663 per mouse per day in the diet for a period of fifteen days. At the end of this period the drug was stopped and the animals were infected intracorneally with a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis*. Another group of 6 un-

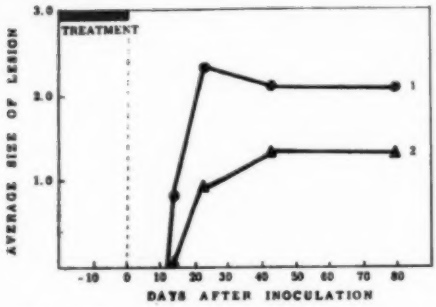

FIG. 4. Showing the development of corneal lesions in mice infected with a 10^{-3} dilution of a culture of *M. tuberculosis* var. *bovis* and treated prior to infection with B663. (*1*) untreated—controls; (*2*) treated with B663—0.3 mg. per mouse per day for two weeks before infection.

treated mice were similarly inoculated with an identical dose of organisms, and this group was included as a control. These mice were observed for a period of twelve weeks.

Results: Administration of B663 prior to infection seems to produce some beneficial effect, as was seen by the increase in the incubation period of the disease in the treated group (figure 4). Two of 12 animals treated with B663 developed macroscopic corneal lesions by the thirteenth day, whereas all of the animals in the control group had already developed macroscopic corneal lesions at that time. By the twenty-second day, 7 more animals of the treated group developed corneal lesions; in 2 animals no macroscopic lesion developed at all during the period of observation, and in one a tiny lesion developed but resolved completely later on. However, when macroscopic corneal lesions did appear in the treated animals, they usually developed to a size similar to those in control animals.

Thus, it would seem that, when the drug is given before infection, in the dosage used here, there is a delaying effect on the development of tuberculous lesions in the cornea, and there may even be complete suppression of lesion formation.

DISCUSSION

The present findings confirm those of Barry and his co-workers in showing that the phenazine derivative B663 is highly effective in experimental tuberculosis in mice: a dose as low as 2 mg. per kg. per day produced some decrease of the corneal lesions, as compared with untreated controls. The higher daily dose of 12 mg. per kg. per day did not produce complete inhibition of the tuberculous process in the cornea, although such a dose of isoniazid will, as a rule, completely prevent bacterial multiplication and the formation of any macroscopic lesion during the period of treatment. It is difficult to compare the chemotherapeutic effectiveness of two drugs with such different pharmacologic properties, i.e., isoniazid, which is rapidly cleared from the body, and B663 which, as shown by Barry and co-workers (2) and confirmed in the present investigation, has marked cumulative properties. The results in this laboratory with B663 seem, however, to be rather less impressive than those of Barry. To some extent the effects of the phenazine compound are qualitatively rather similar to those obtained with streptomycin using the corneal technique (4). In both cases there is marked inhibition, but not complete suppression, of the macroscopic tuberculous lesion in the cornea. The comparative ineffectiveness of streptomycin in such experiments is probably due to the failure of the drug to penetrate well into the macrophages which take up the bacilli, but this does not apply to B663, since the globules of dye actually inside the macrophages could be seen.

It must, therefore, be emphasized that the present experiments have shown that the phenazine derivative, B663, is a highly active antituberculous drug, which cumulates in the body and probably in the corneal tuberculous lesion.

SUMMARY

The antituberculous activity of the phenazine derivative B663 was investigated both *in vitro* and *in vivo*. It was shown to have bactericidal activity in a concentration of 0.8γ per ml. and to be taken up by the bacilli from the medium.

In vivo, using mouse corneal infection with *M. tuberculosis* var. *bovis*, the phenazine was shown to have some effect in a dose as low as 2 mg. per kg. daily. A dose of 12 mg. per kg. daily combined with 12 mg. of isoniazid per kg. did not produce any better effect than that dosage of isoniazid alone. The phenazine derivative was also shown to accumulate in the tissues and probably in the tuberculous corneal lesion and to remain in the tissues for some time after cessation of treatment.

Sumario

Actividad Antituberculosa de un Derivado de la Fenacina (B663)

La actividad antituberculosa del derivado de la fenacina, B663, fué investigada tanto *in vitro* como *in vivo*. Se observó que poseía actividad bactericida a una concentración de 0.8γ por ml. y que era absorbido por los bacilos del medio.

In vivo, usando la infección corneal del ratón por el *M. tuberculosis* var. *bovis*, se observó que la fenacina ejercía algún efecto a una dosis que no pasaba de 2 mg. diarios por kg. Una dosis diaria de 12 mg. por kg. combinada con 12 mg. de isoniacida por kg. no produjo mejor efecto que la misma dosis de isoniacida sola. Se observó además que el derivado de la fenacina se acumulaba en los tejidos y probablemente en la lesión tuberculosa de la córnea y permanecía en los tejidos por algún tiempo después del cese del tratamiento.

Resume

Activité antituberculeuse d'un dérivé de la phénazine (B663)

L'activité antituberculeuse du dérivé B663 de la phénazine a été étudiée *in vitro* et *in vivo*. On a constaté que ce dérivé avait une activité bactéricide à la concentration de 0.8γ par ml. et qu'il était absorbé par les bacilles à partir du milieu.

In vivo, en infectant la cornée de la souris par *M. tuberculosis var. bovis*, il a été prouvé que la phénazine agissait à une dose quotidienne aussi peu élevée que 2 mg. par kg. Une dose de 12 mg. par jour par kg., combinée à 12 mg. d'isoniazide par kg., n'a pas produit de meilleur résultat que la même dose d'isoniazide administrée seule. Il a été aussi constaté que le dérivé de la phénazine s'accumulait dans les tissus et probablement dans la lésion tuberculeuse cornéenne et qu'il persistait dans les tissus pendant quelque temps après la fin du traitement.

Acknowledgment

The writers wish to thank Professor V. C. Barry, and Mr. Bavin of Smith & Nephew Ltd., for the supplies of B663.

REFERENCES

(1) Barry, V. C., Belton, J. G., Conalty, M. L., Denneny, J. M., Edward, D. W., O'Sullivan, J. F., Twomey, D., and Winder, F.: A new series of phenazines (Rimino-compounds) with high anti-tuberculosis activity, Nature, London, 1957, *179*, 1013.

(2) Barry, V. C., and Conalty, M. L.: Antituberculosis activity in the phenazine series, Am. Rev. Tuberc., 1958, *78*, 62.

(3) Rees, R. J. W., and Robson, J. M.: Experimental tuberculous infections of the cornea of the mouse: A screening test for antituberculous substances, Brit. J. Pharmacol., 1950, *5*, 77.

(4) Goulding, R., and Robson, J. M.: Isoniazid in the control of experimental corneal tuberculosis, Lancet, 1952, *2*, 849.

(5) Acharya, B. K., Sullivan, F. M., and Robson, J. M.: Immunity in experimental tuberculosis, Am. Rev. Tuberc., 1958, *78*, 203.

A COMPARISON OF THE VIRULENCE OF VARIOUS KNOWN AND ATYPICAL MYCOBACTERIA FOR CHICKENS, GUINEA PIGS, HAMSTERS, AND MICE[1, 2]

FREDERICK E. DURR, DONALD W. SMITH, AND DAVID P. ALTMAN

(Received for publication April 13, 1959)

INTRODUCTION

Interest in the role of atypical mycobacteria as a cause of disease in man was first stimulated through the reports of Timpe and Runyon (1) and Buhler and Pollak (2). More recent studies of Lewis and associates (3) and especially of Keltz, Colton, and Lester (4) suggest an even greater incidence of infection due to this group of mycobacteria than was heretofore realized. The classification proposed by Runyon (5), based on pigment formation on laboratory media, is the most widely accepted means of subgrouping the atypical mycobacteria.

The feature possessed by the atypical strains that differentiates them from true mammalian strains is their inability to produce progressive disease in guinea pigs. On the other hand, the photochromogenic atypical mycobacteria have been shown to be uniform in their ability to produce extensive disease in hamsters and mice. Reports of disease production by nonphotochromogenic and scotochromogenic strains are less frequent, but in general these latter two types are less virulent for all of the species tested.

Only a few very limited studies on the virulence of atypical mycobacteria for chickens have appeared in the literature. Buhler and Pollak (6) found that their "yellow bacillus" failed to produce progressive disease in chickens. Feldman and his co-workers (7), and more recently Engback and Ohlsen (8), have isolated from human disease acid-fast organisms which resemble avian tubercle bacilli in many ways but which fail to produce characteristic, progressive disease in chickens. Runyon (9), in a recent review of atypical mycobacteria, indicated that these two strains most closely resembled nonphotochromogens, and he placed them with Group III organisms.

Solotorovsky and associates (10) reported in 1957 a study of representative cultures of the three types of atypical mycobacteria in chickens. He tested strains of the collection of the Laboratory Subcommittee of the American Trudeau Society (strains 5, 7, 17, 23, 18, and 22) for their ability to produce disease in three-week-old chickens, and found only one (strain 23) to be pathogenic. It has since been reported (11) that culture 23 of this collection may be a mixture of photochromogenic and nonphotochromogenic bacilli, and for this reason it would be difficult to associate the disease-producing capacity with either of these two types of bacilli.

The interest of the present writers in an evaluation of the virulence of atypical acid-fast bacilli for chickens comes from three sources. First, the appearance of nonphotochromogenic cultures on American Trudeau Society medium bears certain resemblances to avian strains. Second, it has been demonstrated by Froman (12) through phage typing that both avian strains and most nonphotochromogens are resistant to lysis by mycobacteriophage. The third observation calling attention to the similarity of avian strains and nonphotochromogens is the report by Smith and co-workers (13) of the presence of a specific lipid substance occurring only in avian strains and in nonphotochromogenic bacilli. This fraction, referred to as "Jav," appears to be a glycolipid-peptide and is closely related to another fraction, "Jat," found only in atypical bacilli of the scotochromogenic type. A detailed report on the distribution of strain specific lipids in mycobacteria will appear soon (14).

On the basis of these three observations, it seemed desirable to study the virulence of various types of atypical acid-fast bacilli in guinea pigs, mice, hamsters, and chickens. Greatest attention was given to comparative studies of atypical and

[1] From the Department of Medical Microbiology, University of Wisconsin, Madison, Wisconsin.

[2] Supported by grants from the Wisconsin Alumni Research Foundation, and from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

avian strains in chickens. Control groups of animals were injected with standardized inocula of *M. tuberculosis*, *M. avium*, *M. phlei*, and *M. smegmatis*.

In general, the results of this study confirm those of earlier writers in demonstrating that photochromogenic atypical bacilli are more virulent for hamsters and mice than for guinea pigs, and that nonphotochromogenic strains are less virulent for all species. It was not possible to demonstrate the development of progressive disease in chickens with any strain other than *M. avium*.

Material and Methods

Animals: The chickens inoculated with *M. tuberculosis*, *M. avium*, *M. phlei*, and *M. smegmatis* in *Experiment I* were White Leghorn hens. White Leghorn pullets or cockerels, three to six weeks old, were used in all of the subsequent investigations. The chickens were routinely tuberculin tested with 0.05–0.10 ml. of avian tuberculin prior to infection. No positive reactors were detected.

White Swiss-Webster mice (virus susceptible and Salmonella resistant) bred and maintained by the Department of Medical Microbiology were used in all of the studies. Adult Syrian Golden Hamsters and six-week-old guinea pigs were also used in the present investigation.

Cultures: Stock cultures of mycobacteria maintained on American Trudeau Society medium were transferred to Dubos Tween®-albumin liquid medium. Human, avian, and atypical strains were incubated at 37°C. for five to seven days, and *M. phlei* and *M. smegmatis* at 37°C. for two days prior to animal inoculation. Viable plate counts on appropriate dilutions of the cultures used as inocula were determined by the method of Fenner and Martin (15), and the number of viable units per ml. was calculated and is recorded in tables 2–6.

The cultures used in the present study and their sources are indicated in table 1.

Animal inoculation: Tween-albumin broth cultures were appropriately diluted for use as inocula. The chickens used in *Experiment I* (two per culture) were injected subcutaneously with 1.0 ml. of diluted culture, and the majority in *Experiment II* were injected intravenously with 0.4 ml. of the diluted cultures. Five guinea pigs received 1.0 ml. subcutaneously; 3 hamsters, 1.0 ml. intraperitoneally; and 10 mice, 0.4 ml. of the diluted cultures intravenously.

Prior to sacrifice, surviving chickens and guinea pigs were tuberculin tested with 0.05 to 0.10 ml. of undiluted avian tuberculin and intermediate-strength mammalian tuberculin (PPD), respectively. Eight weeks after infection the animals were killed and examined. Observations of gross disease were made at necropsy, and portions of spleen were removed for culture purposes. Splenic homogenates were treated by the sodium hydroxide method, and the neutralized sediment was

TABLE 1

Cultures Used in the Present Study and Their Sources

Culture	Source
M. tuberculosis	
Strain 199	National Institutes of Health: B. J. Olson
*M. avium**	
297	Mayo Foundation: W. H. Feldman
298	Mayo Foundation: W. H. Feldman
299	Mayo Foundation: W. H. Feldman
300	Mayo Foundation: W. H. Feldman
Faisan II	Pasteur Institute: E. Lederer
Fonteau	Pasteur Institute: E. Lederer
10908	Animal Disease Laboratory, Madison, Wisconsin
1052B	Animal Disease Laboratory, Madison, Wisconsin
10435	Animal Disease Laboratory, Madison, Wisconsin
Atypical mycobacteria	
Photochromogens† 1, 4, 16, 22	Laboratory Subcommittee, American Trudeau Society: E. H. Runyon
Nonphotochromogens 3, 7, 20, and 23	Laboratory Subcommittee, American Trudeau Society: E. H. Runyon
Saprophytes	
M. phlei	Rutgers University: Ruth Gordon
M. smegmatis	Rutgers University: Ruth Gordon

* Avian mycobacteria will be hereafter referred to as Av-297, Av-Fonteau, et cetera.

† Photochromogens will be hereafter indicated by PH-1, PH-4, et cetera, and nonphotochromogens as N-PH-7, N-PH-20, et cetera.

cultured on two American Trudeau Society slants. Portions of liver and spleen from chickens, and lung, liver, spleen and occasionally kidney from the other animals were fixed in 10 per cent formalin. Tissue sections were subsequently made and stained with hematoxylin and eosin and by the Ziehl-Neelsen or Aubert method.

Results

Experiment I: Pathogenicity of known and atypical mycobacteria for a selected group of animals. The results of chicken, guinea pig, hamster, and mouse inoculation with known and atypical strains of mycobacteria are recorded in tables 2–5. The tables summarize the disease process as it occurred in the experimental animals and is presented in greater detail elsewhere (16).

Gross disease was recorded as negative, minimal, or extensive, and microscopic pathology was described as nonspecific change, early tuberculosis, granulomatous tuberculosis, or granulomas with caseation. Negative for gross disease refers to the absence of demonstrable lesions or enlargement of the liver or spleen. Minimal disease was considered to exist if there was evidence of liver and splenic enlargement with or without the presence of a few lesions. Extensive gross disease refers to the presence of numerous macroscopic lesions on lungs, liver, or spleen with the usual enlargement of the latter two organs.

Nonspecific change refers to tissue changes in response to foreign or toxic agents which are not particularly characteristic of tuberculosis. Included in this category were alveolar thickening, fatty vacuolization of hepatic cells, perivascular

TABLE 2

Infection of Chickens with Known and Atypical Mycobacteria

Culture	Viable Units per Ml.*	Gross Disease	Histopathology	Presence of Acid-fast Bacilli	Recovery on Culture of Spleen
Part A†					
M. tuberculosis	5.0×10^6	Negative	Negative	Negative	Negative
Av. 297	5.0×10^6	CH-1—negative CH-2—died	Granulomas Slight caseation	CH-1—negative CH-2—sparse	CH-1—negative CH-2—positive
M. phlei	1.0×10^7	Negative	Negative	Negative	Negative
M. smegmatis	1.0×10^7	Negative	Negative	Negative	Negative
N-PH 20	1.0×10^7	Negative	Negative	Negative	Negative
PH-4	1.0×10^7	Negative	Negative	Negative	Negative
PH-21	1.0×10^7	Negative	Negative	Negative	Negative
PH-1	1.0×10^7	CH-1—negative CH-2—minimal	CH-1—Early tuberculosis CH-2—Small granulomas	Negative	Negative
PH-16	1.0×10^7	Negative	Negative	Negative	Negative
Part B‡					
N-PH 20	2.0×10^7	Negative	Small granulomas	Sparse	Positive
PH-21	2.0×10^6	Negative	Small granulomas	Negative	Negative
Av. 300	2.0×10^7	Minimal; one died	Granulomas with caseation	Numerous	Positive

* Each of 2 chickens received *1.0 ml.* of diluted culture subcutaneously with the number of organisms per ml. indicated in the table. The results tabulated are based upon total numbers of animals except where indicated.

† Part A—Infection of White Leghorn hens with known and anonymous strains.

‡ Part B—A limited repeat study using representative organisms and 6-week-old White Leghorn pullets.

PH—represents photochromogen.

N-PH—represents nonphotochromogen.

Av.—represents avian strains.

CH-1 or CH-2 refers to either one of the two chickens inoculated with the various mycobacteria.

TABLE 3
INFECTION OF GUINEA PIGS WITH KNOWN AND ATYPICAL MYCOBACTERIA

Culture	Viable Units per Ml.*	Gross Disease	Histopathology	Presence of Acid-fast Bacilli	Recovery on Culture of Spleen
Part A†					
M. tuberculosis	5.0×10^6	Extensive; all died	Not done	Numerous	Not done
Av. 297	5.0×10^6	Negative	Nonspecific changes	Negative	Negative
M. phlei	1.0×10^7	Negative	Negative	Negative	Negative
M. smegmatis	1.0×10^7	Negative	Negative	Negative	3 of 5 positive
N-PH 20	1.0×10^7	Negative	Nonspecific changes	Negative	2 of 4 positive
PH-4	1.0×10^7	Minimal	Nonspecific changes	Negative	1 of 5 positive
PH-21	1.0×10^7	Negative	Nonspecific changes	Negative	Negative
PH-1	1.0×10^7	Minimal	Nonspecific changes	Negative	Negative
PH-16	1.0×10^7	Minimal	Nonspecific changes	Negative	Negative
Part B‡					
N-PH 20	2.0×10^7	Negative	Negative	Negative	Not done
PH-21	2.0×10^6	Minimal	Nonspecific changes	Negative	Negative
Av. 300	2.0×10^7	Minimal	Negative	Negative	Positive

* Each of 5 guinea pigs received as an inoculum 1.0 ml. of the diluted culture containing the number of organisms per ml. indicated in the table. The results tabulated are based upon total numbers of animals except where indicated.

† Part A—Infection of guinea pigs by the subcutaneous route with known and atypical strains.

‡ Part B—A limited repeat study using representative organisms inoculated intraperitoneally into guinea pigs.

PH—represents photochromogen.

N-PH—represents nonphotochromogen.

Av.—represents avian strains.

leukocytic infiltration, and hyperemia. "Early tuberculosis" was used to describe tissues which contained acid-fast bacilli but which did not show progression to epithelioid tubercles. Microscopic lesions described as granulomas refer to circumscribed regions composed of epithelioid cells with peripherally located lymphocytes and, occasionally, fibroblasts. These have been described as either small or large granulomas and are considered the same as epithelioid or "hard" tubercles. If the central portion of the granulomas had undergone caseous necrosis, the lesion was described simply as a granuloma with caseation.

Atypical acid-fast organisms produced very little evidence of disease in chickens and guinea pigs. The photochromogens did, however, produce extensive disease in hamsters and mice, whereas the nonphotochromogen (strain 20) produced very slight, if any, disease in any of the test animals. Although *M. tuberculosis* failed to produce the extensive disease expected in mice, it did produce characteristic disease in guinea pigs and hamsters: all of these animals died within the test period. *Mycobacterium avium* was found to cause some degree of disease in all of the animals, but the macroscopic and microscopic involvement found in hamsters and mice did not approach the extensive disease produced by the photochromogens.

Experiment II: An extended investigation comparing the virulence of avian and atypical strains of mycobacteria for chickens. The results of this experiment may be seen in table 6. It is immediately evident that known avian strains of mycobacteria produce extensive, progressive disease in chickens under the conditions of this study. At the time of sacrifice, the livers and spleens were grossly involved, and microscopic examination invariably revealed large caseous granulomas. The lesions were filled with acid-fast bacilli, and in some instances it might be said that the tissue was replaced by acid-fast organisms.

In striking contrast to this very extensive involvement due to avian mycobacteria was the degree of disease produced by the atypical acid-fast organisms in which gross findings were negligible and microscopic disease, when present, was indicated by small circumscribed granulomas without caseation. Acid-fast bacilli were not detected in the tissues nor could they (with the

TABLE 4

INFECTION OF HAMSTERS WITH KNOWN AND ATYPICAL MYCOBACTERIA

Culture	Viable Units per Ml.*	Gross Disease	Histopathology	Presence of Acid-fast Bacilli	Recovery on Culture of Spleen
Part A†					
M. tuberculosis	5.0×10^6	Extensive; all died	Nonspecific changes; small granulomas	Numerous	Not done
Av. 297	5.0×10^6	Negative	Granulomas	Sparse	Positive
M. phlei	1.0×10^7	Negative	Negative	Negative	Negative
M. smegmatis	1.0×10^7	Negative	Negative	Negative	Negative
N-PH 20	1.0×10^7	Negative	Nonspecific changes and small granulomas	H_1 and $_2$—negative; H_3—sparse	Positive
PH-4	1.0×10^7	Minimal	Granulomas	Numerous	Positive
PH-21	1.0×10^7	Extensive	Granulomas	Numerous	Positive
PH-1	1.0×10^7	Extensive	Granulomas	Numerous	Positive
PH-16	1.0×10^7	Extensive	Granulomas	Numerous	Positive
Part B‡					
N-PH 20	2.0×10^7	Negative	Nonspecific changes	Negative	Positive
PH-21	2.0×10^6	Extensive	Granulomas; some calcification	Negative	Positive
Av. 300	2.0×10^7	Negative	Not done	Negative	Not done

* Each of 3 hamsters received as an inoculum 1.0 ml. of the diluted culture containing the number of organisms per ml. indicated in the table. The results tabulated are based upon total numbers of animals except where indicated.

† Part A—Infection of hamsters by the intraperitoneal route with known and atypical strains.

‡ Part B—A limited repeat study using representative organisms.

PH—represents photochromogen.

N-PH—represents nonphotochromogen.

A.—represents avian strains.

H_1 or H_2, et cetera, refers to one particular hamster inoculated with various mycobacteria.

TABLE 5

INFECTION OF MICE WITH KNOWN AND ATYPICAL MYCOBACTERIA

Culture	Viable Units per Ml.*	Gross Disease	Histopathology	Presence of Acid-fast Bacilli	Recovery on Culture of Spleen
Part A†					
M. tuberculosis	5.0×10^6	Minimal	Early tuberculosis; nonspecific changes	Numerous	Positive
Av. 297	5.0×10^6	Negative	Granulomas	Numerous	Positive
M. phlei	1.0×10^7	Negative	Negative	Negative	Negative
M. smegmatis	1.0×10^7	Negative	Negative	Negative	Positive
N-PH 20	1.0×10^7	Negative	Negative	Sparse	Positive
PH-4	1.0×10^7	Extensive	Granulomas	Numerous	Positive
PH-21	1.0×10^7	Extensive	Granulomas	Numerous	Positive
PH-1	1.0×10^7	Extensive	Granulomas	Numerous	Positive
PH-16	1.0×10^7	Extensive	Granulomas	Numerous	Positive
Part B‡					
N-PH 20	2.0×10^7	Negative	Negative	Negative	Positive
PH-21	2.0×10^6	Extensive	Granulomas	Negative	Positive
Av. 300	2.0×10^7	Extensive	Granulomas	Numerous	Not done

* Each of 10 mice received as an inoculum 0.40 ml. of diluted culture containing the number of organisms per ml. indicated in the table. The results tabulated are based upon total numbers of animals except where indicated.

† Part A—Infection of Swiss-Webster mice by the intravenous route with known and atypical strains.

‡ Part B—A limited repeat study using representative organisms.

PH—represents photochromogen.

N-PH—represents nonphotochromogen.

Av.—represents avian strains.

TABLE 6

INFECTION OF CHICKENS* WITH AVIAN AND ATYPICAL MYCOBACTERIA

Culture	Viable Units per Ml.†	Gross Disease	Histopathology	Presence of Acid-fast Bacilli	Recovery on Culture of Spleen
Av. 297	4.0×10^5	Extensive	Granulomas with caseation	Teeming	Positive
Av. 298	3.5×10^5	Extensive	Granulomas with caseation	Numerous	Positive
Av. 299	3.0×10^5	Extensive	Granulomas with caseation	Teeming	Positive
Av. 300‡	6.0×10^5	Extensive	Granulomas with caseation	Teeming	Positive
Av. Faisan II	9.0×10^5	Extensive	Granulomas with caseation	Teeming	Positive
Av. Fonteau	4.0×10^5	Extensive	Granulomas with caseation	Teeming	Positive
Av. 10908	7.0×10^5	Extensive	Granulomas with caseation	CH-1—sparse; CH-2—numerous	Positive
Av. 1052B‡	2.0×10^6	Extensive	Granulomas with caseation	Teeming	Positive
Av. 10435	1.5×10^6	Minimal	Granulomas with caseation	Numerous	Positive
PH-1	4.5×10^5	Negative	Small granulomas	Negative	Negative
PH-4	7.5×10^5	CH-1—minimal; CH-2—negative	Small granulomas	Negative	CH-1—positive; CH-2—negative
PH-16	9.0×10^5	CH-1—negative; CH-2—positive	CH-1—negative; CH-2—small granulomas	Negative	Negative
PH-22	5.0×10^5	Negative	Negative	Negative	Negative
N-PH 3	1.0×10^6	Negative	Negative	Negative	Negative
N-PH 7	7.0×10^5	Negative	Small granulomas	Negative	Negative
N-PH 20‡	5.0×10^5	Negative	Small granulomas	Negative	Negative
N-PH 23‡	2.0×10^6	Negative	Negative	Negative	Negative

* White Leghorn pullets and cockerels, 3 to 4 weeks old, were used in this experiment.

† Each of 2 chickens received as an inoculum 0.40 ml. of diluted culture (intravenous except where indicated by a ‡, then subcutaneous) containing the number of organisms per ml. indicated in the table. The results tabulated are based upon total numbers of animals except where indicated.

PH—represents photochromogen.

N-PH—represents nonphotochromogen.

Av.—represents avian strains.

exception of one positive culture) be recovered by culture of splenic homogenates.

The differences in lesions produced by avian and atypical mycobacteria are indicated in figures 1 and 2. Any differences in the extent of disease produced in this experiment and in those observed with avian organisms in *Experiment I* are most likely attributable to the different route of inoculation (table 7).

Tuberculin tests with avian tuberculin were done on chickens infected with avian and atypical mycobacteria; however, the results were irregular and inconclusive.

Discussion

The contributions made by this study to the results already reported by Timpe and Runyon (1), Buhler and Pollak (6), and Solotorovsky and associates (10) are threefold. The comparison in one experiment of the virulence of various types of mycobacteria in guinea pigs, mice, hamsters, and chickens allows a better evaluation of the relative virulence of these strains.

Second, the determination of the challenge inoculum in terms of viable units of the various strains used gives a more accurate basis for com-

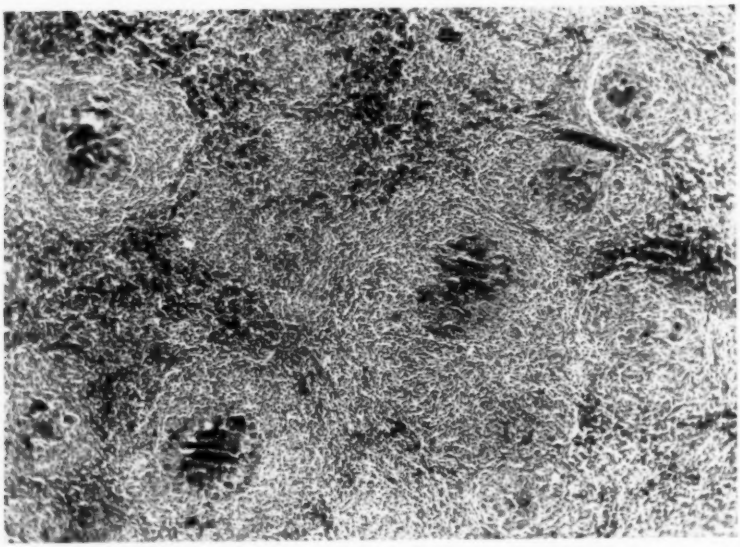

FIG. 1. Microscopic section of chicken spleen showing the extensive tissue damage produced by *M. avium*. A granulomatous tissue response with central caseous necrosis characterizes this typical splenic involvement. (Hematoxylin and eosin stain, ×100) Masses of acid-fast bacilli were found in these lesions when stained by the Aubert method.

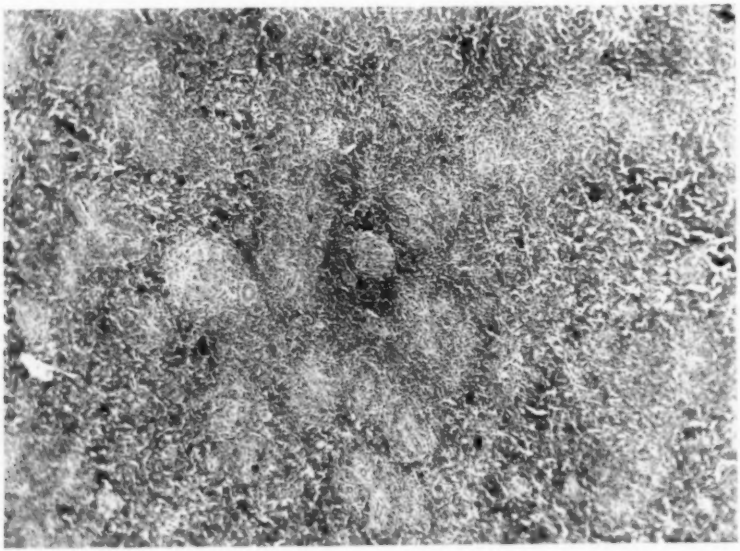

FIG. 2. Microscopic section of chicken spleen showing the maximal tissue involvement produced by a photochromogen. The few lesions that are present are epithelioid tubercles. Note tubercle left of center. (Hematoxylin and eosin stain, ×100) No acid-fast bacilli could be detected in these lesions when stained by the Aubert method. Nonphotochromogens were found to produce the same, and frequently less, tissue involvement than photochromogens.

TABLE 7

SUMMARY OF DATA PRESENTED COMPARING THE PATHOGENICITY OF VARIOUS MYCOBACTERIA FOR THE SPECIES OF ANIMALS TESTED

	Chicken	Guinea Pig	Hamster	Mouse
M. tuberculosis	−	+++	+++	+
M. avium	++	−	+	+
Nonphotochromogens	±	−	±	−
Photochromogens	±	−	++	++
Saprophytes				
M. phlei	−	−	−	−
M. smegmatis				

Progressive disease resulting in death of all of the animals in a particular group is indicated by +++; extensive gross disease without a fatal outcome by ++; microscopic disease only, by +; and microscopic disease in some, but not all, of the animals in a particular group, by ±.

One strain each of *M. phlei*, *M. smegmatis*, and *M. tuberculosis* was used for the inoculation of chickens, guinea pigs, hamsters, and mice. One nonphotochromogenic and three photochromogenic strains were used for inoculation of guinea pigs, hamsters, and mice. Nine avian strains, four photochromogenic, and four nonphotochromogenic strains were tested in chickens.

paring the results of these experiments with the results of experiments by other investigators in which the challenge inoculum is determined on the same basis. Finally, inclusion of similar inocula of known strains of nonvirulent mycobacteria (*M. phlei* and *M. smegmatis*) is a control on the extent of pathology produced by these organisms.

The first experiment, in which chickens, guinea pigs, hamsters, and mice were infected with known and atypical strains of mycobacteria, is essentially a preliminary experiment. The results largely substantiate the observations of a number of other workers and give an indication of the animal pathogenicity of the organisms being used for the present study.

The second experiment was designed to offer a more critical examination of animal pathogenicity by comparing the virulence of avian and atypical mycobacteria for chickens. The results indicate that there is a striking difference between the extent of disease caused by avian mycobacteria and that produced by the atypical acid-fast bacilli. This finding would not support a contention that atypical mycobacteria, and especially the nonphotochromogens, are closely related to virulent avian mycobacteria. The possibility of the atypical organisms being attenuated avian strains is, of course, not excluded by the present study.

The reasons for considering a relationship of avian and nonphotochromogenic mycobacteria have been cited previously. The most suggestive evidence relating the two organisms has been provided by Froman (12) and Smith and his co-workers (13). Froman has observed a resistance of nonphotochromogens and avian tubercle bacilli to lysis by mycobacteriophage. Photochromogens have been found susceptible to lysis by phage and are thus distinguished from the above organisms. Smith and Randall, utilizing methods of chromatography and infrared spectroscopy, have found still another similarity between nonphotochromogens and avian mycobacteria. They have isolated and spectroscopically characterized a specific glycolipid-peptide, designated "Jav," from some avian strains and from nonphotochromogens.

This evidence, suggesting a relationship between nonphotochromogens and some avian tubercle bacilli, is neither rejected nor augmented on the basis of animal pathogenicity studies. However, nonphotochromogens were found to be much less virulent than avian strains for the chicken as well as for the other experimental animals used in this investigation. This lower virulence may account for the frequent finding of nonphotochromogens in human disease in which pathogenicity appears to be enhanced by some predisposing factor. Thus, the organisms showing perhaps the greatest resemblance based on all of the available data are the most dissimilar when their pathogenicity is measured. A link between avian and atypical mycobacteria could have been furnished by nonphotochromogens, but such a relationship has not been supported by the study presented herein.

Since different methods and courses of investigation have yielded evidence relating atypical mycobacteria in one instance to virulent types such as *M. tuberculosis* and *M. avium* and, in the other, to saprophytic types such as *M. phlei* or *M. smegmatis*, it would seem best at the present time to consider the atypical mycobacteria as an independent group of organisms with no obvious relationship to either virulent mammalian, avian, or saprophytic mycobacteria.

SUMMARY

Various strains of mycobacteria were compared for their capacity to produce disease in a selected

group of animals. Special attention was directed toward a comparison of avian and atypical mycobacteria, using the chicken as the host. The results of this study are in accord with the observations of other investigators regarding the pathogenicity of human, avian, saprophytic, and atypical mycobacteria for chickens, guinea pigs, hamsters, and mice.

Mycobacterium tuberculosis and *M. avium* produced typical progressive disease in the respective susceptible animals. Saprophytes did not produce any demonstrable disease in the animals, but *M. smegmatis* was recovered from the spleens of a number of mice and guinea pigs.

The photochromogens regularly caused progressive disease in hamsters and mice, although none died of the infection within the eight-week test period. Photochromogens and nonphotochromogens produced very little disease, if any, in the chicken, whereas avian tubercle bacilli produced extensive disease in this animal. Nonphotochromogens were much less virulent for any of the test animals than were the photochromogens.

The results of this animal pathogenicity study suggest that, if atypical mycobacteria and especially nonphotochromogens are related to avian mycobacteria, they are most certainly very attenuated avian strains.

The findings of this investigation are discussed in relation to the observations of other workers.

Sumario

Comparación de la Virulencia de Varias Micobacterias Conocidas y Atípicas para Pollos, Cobayos, Cricetos y Ratones

Se compararon varias cepas de micobacterias en cuanto a su capacidad patógena en un grupo seleccionado de animales. Se prestó atención en particular a una comparación de micobacterias aviarias y atípicas, usando el pollo como huésped. Los resultados de este estudio convienen con las observaciones de otros investigadores con respecto a la patogenicidad de las micobacterias humanas, aviarias, saprofitas y atípicas para pollos, cobayos, cricetos y ratones.

El *Mycobacterium tuberculosis* y el *M. avium* produjeron típica enfermedad evolutiva en los respectivos animales susceptibles. Los saprofitos no produjeron ninguna enfermedad observable en los animales, pero se obtuvo el *M. smegmatis* de los bazos de varios ratones y cobayos.

Los fotocromógenos provocaron con regularidad enfermedad evolutiva en los cricetos y los ratones, aunque ninguno de éstos sucumbió de la infección en el período de ocho semanas de ensayo. Los fotocromógenos y los anafotocromógenos produjeron muy poca, o ninguna, enfermedad en el pollo, en tanto que los bacilos tuberculosos aviarios produjeron extensa enfermedad en este animal. Los anafotocromógenos fueron mucho menos virulentos que los cromógenos para todos los animales de ensayo.

Sugieren los resultados de este estudio de la patogenicidad para los animales que, si las micobacterias atípicas y en particular las anafotocromógenas están relacionadas con las aviarias, son con toda certeza cepas aviarias muy atenuadas.

Se discuten los hallazgos de esta investigación en relación con las observaciones de otros técnicos.

Resume

Comparaison de la virulence de diverses mycobactéries connues et atypiques chez la poule, le cobaye, l'hamster et la souris

Le pouvoir pathogène sur certains animaux de diverses souches de mycobactéries a été comparé. En utilisant la poule comme hôte, les mycobactéries aviennes et atypiques ont été étudiées avec grand soin. Les résultats de ces études correspondent aux observations faites par d'autres auteurs en ce qui concerne le pouvoir pathogène des mycobactéries humaines, aviennes, saprophytiques et atypiques chez la poule, le cobaye, l'hamster et la souris.

Le *mycobacterium tuberculosis* et le *M. avium* ont produit une affection progressive typique chez les divers animaux sensibles. Des saprophytes n'ont produit aucune maladie apparente chez ces animaux, mais *M. smegmatis* fut retrouvé dans la rate d'un certain nombre de souris et de cobayes.

Les souches photochromogènes ont produit avec régularité, une maladie progressive chez l'hamster et la souris, bien qu'aucun animal n'ait succombé à l'infection pendant les huit semaines de l'épreuve. Les souches photochromogènes et celles qui n'étaient pas photochromogènes n'ont produit qu'une maladie très atténuée chez la poule, et parfois même aucune réaction, alors que les bacilles tuberculeux aviens causaient une maladie grave chez cet animal. Les souches non-photochromogènes étaient beaucoup moins virulentes chez tous les animaux inoculés que les souches photochromogènes.

Les résultats de cette étude du pouvoir pathogène chez l'animal indiquent que si les mycobactéries atypiques—et plus particulièrement les mycobactéries non-photochromogènes—sont ap-

parentées aux mycobactéries aviennes, ce sont très certainement des souches aviennes très atténuées.

Les résultats de cette étude sont comparés aux travaux d'autres auteurs.

REFERENCES

(1) Timpe, A., and Runyon, E. H.: Relationship of "atypical" acid-fast bacteria to human disease, J. Lab. & Clin. Med., 1954, *44*, 202.

(2) Buhler, V. B., and Pollak, A.: Human infection with atypical acid-fast organisms, Am. J. Clin. Path., 1953, *23*, 363.

(3) Lewis, A. G., Jr., Dunbar, F. P., Lasche, E. M., Bond, J. O., Lerner, E. N., Wharton, D. J., Hardy, A. V., and Davies, R.: Chronic pulmonary disease due to atypical mycobacterial infections, Am. Rev. Resp. Dis., 1959, *80*, 188.

(4) Keltz, H., Colton, R., and Lester, W.: Studies on atypical acid-fast organisms obtained from patients with pulmonary tuberculosis, Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, 1957, p. 290.

(5) Runyon, E. H.: Mycobacteria encountered in clinical laboratories, Leprosy Briefs, 1958, *9*, 21.

(6) Buhler, V. B., and Pollak, A.: The cultural characteristics and animal pathogenicity of an atypical acid-fast organism which causes human disease, Am. Rev. Tuberc., 1955, *71*, 74.

(7) Feldman, W. H., Davies, R., Moses, H. E., and Andberg, W.: An unusual Mycobacterium isolated from sputum of a man suffering from pulmonary disease of long duration, Am. Rev. Tuberc., 1943, *48*, 82.

(8) Engbaek, T. F., and Ohlsen, A. S.: A case of lung disease caused by an atypical acid-fast organism, Acta. tuberc. scandinav., 1957, *34*, 145.

(9) Runyon, E. H.: Anonymous mycobacteria in pulmonary disease, M. Clin. North America, 1959, *43*, 273.

(10) Solotorovsky, M., Stoerk, H. C., and Ironson, E. J.: Virulence of atypical acid-fact organisms for mice; paper presented at a symposium on atypical mycobacteria, *Session 2B*, 53rd annual meeting of the American Trudeau Society, 1957.

(11) Runyon, E. H.: Personal communication.

(12) Froman, S.: Personal communication.

(13) Smith, D. W., Randall, H. M., Gastambide-Odier, M. M., and Koevoet, A. L.: The characterization of mycobacterial strains by the composition of their lipide extracts, Ann. New York Acad. Sc., 1957, *69*, 145.

(14) Randall, H. M., MacLennan, A. P., and Smith, D. W.: Detection of specific lipids in mycobacteria by infrared spectroscopy (in press).

(15) Fenner, F., and Martin, S. P.: The enumeration of viable tubercle bacilli in cultures and infected tissue, Ann. New York Acad. Sc., 1949, *52*, 751.

(16) Durr, F. E.: A comparison of the virulence of known and atypical mycobacteria for experimental animals; thesis submitted in partial fulfillment of requirements for Masters Degree, University of Wisconsin, 1958.

FURTHER STUDIES ON GEL DIFFUSION TESTS IN TUBERCULOSIS[1,2]

I. Methods for Standardization of the Antigen and Detection of Small Amounts of Antibody

ROBERT C. PARLETT AND CAROLYN A. REHR

(Received for publication May 7, 1959)

INTRODUCTION

The results obtained from the testing of 1,097 specimens of serum from tuberculous and 555 specimens from nontuberculous persons using a gel double-diffusion precipitin test have been reported (1). In that study, when an arbitrary means of assessing the degree of positive reactions as 1, 2, 3, and 4-plus was used, it was noted that the majority of positive reactions obtained in testing serum from tuberculous persons were relatively weak. This quasi-quantitative scheme was based on the number of precipitate bands and the amount of precipitate present, according to interpretations derived from two assumptions which frequently appear in the literature (2–6). These assumptions are: (*1*) The total number of precipitate bands observed in a gel diffusion precipitation system is indicative of the minimal number of visible antigen-antibody complex lattice formations present; (*2*) the visibility of the antigen-antibody complex is directly related to the volume of antibody participating in the formation of the complex.

One purpose of this report is to test the validity of the second assumption and consequently the method used for recording the degrees of positive reactions encountered in testing serum from tuberculous persons.

A second purpose of this report is to describe a technique which can be used to increase the sensitivity of the test and, therefore, may assist in overcoming difficulties which might be encountered in practical clinical use of the gel diffusion test because of the predominance of rather low levels of antibody in serum from tuberculous persons.

The antigen mixture used in the gel diffusion test is difficult to standardize (1). Therefore, a new technique is needed which would ensure that the activity of two different antigen preparations would be comparable. One means of accomplishing this end is presented in this paper.

MATERIALS AND METHODS

The methods used were variations of the procedures given in other reports (1, 7). Soft glass tubes, 10 cm. long with an internal diameter of 0.3 mm., were employed. The internal surface of the tubes was coated with a thin film of 0.1 per cent agar (prepared with distilled water) before use.

The first agar layer was prepared by using 2 per cent melted sterile purified agar[3] to dilute antigen or antiserum to the desired concentration. The first agar column, 3.0 cm. in length, was added to the tubes with a sterile Wintrobe pipette.[4] A second layer, 0.75 cm. in depth and consisting of 1 per cent purified agar,[5] was then placed over the first layer. This second agar column contained no reagent and served as the area where antigen-antibody complex formation was observed. When the second had gelled, either serum or antigen, again dependent upon the test situation, was used to fill the remainder of the double-diffusion tube. A schematic illustration of a reaction tube with its component columns is shown in figure 1.

The filled tubes were incubated in a clean covered container (beaker or mason jar) at 37° C. All tests were observed at twenty-four-hour intervals for seven days, and the number of clearly defined precipitate bands in each tube was observed. In some cases the actual number of observed bands was recorded. In other cases in which comparisons were made, however, the reactions were recorded only in terms of 1 to 4-plus (1).

In the replacement technique, used to increase intensity of reactions, fresh reagent was used at regular intervals to replace the material overlying the second agar column. The time of this reagent replacement varied with the individual test situations. Amplification of these techniques will be

[1] From the Department of Microbiology, Northwestern University Medical School, Chicago, Illinois.

[2] Supported by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Prepared with 0.01 molar phosphate buffer of pH 7.5 and with 1.0 molar glycine (CP).

[4] A. S. Aloe and Company, St. Louis, Missouri.

[5] The 2 per cent phosphate buffered agar used in the first column was diluted to 1 per cent with 0.9 per cent saline.

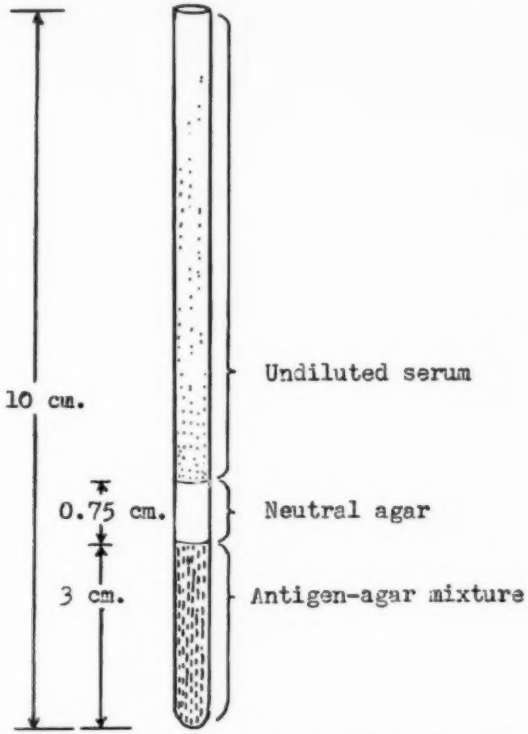

FIG. 1. Diagram of the gel double-diffusion tube showing the relationship between the three component columns

given later in the body of this paper, when the situations to which they apply arise.

The antigens and rabbit antisera employed in these investigations were prepared according to the methods mentioned in previous publications (1, 8). The H37Ra strain of *Mycobacterium tuberculosis* was used for all antigen preparations.

RESULTS

The following experiment was undertaken to determine whether the single precipitate bands recorded as 1-plus reactions in the gel diffusion tests truly were a reflection of the presence of only small amounts of antibody.

Rabbit antiserum which consistently produced 6 separate and distinct bands of precipitate in gel double-diffusion reactions was prepared in 10 twofold dilutions. Each of the 10 dilutions was used as a separate test serum in a gel diffusion test. At the same time, a large number of tests were set up with undiluted specimens of serum from tuberculous persons. All tests were observed and compared at daily intervals throughout the seven days of incubation. Undiluted rabbit antiserum gave a 4-plus reaction characterized by 6 precipitate bands. Only 8.2 per cent of 801 positive tests obtained with serum from tuberculous persons (1) gave reactions similar in appearance to that obtained with undiluted rabbit antiserum. Furthermore, it was noted that, as the dilutions of the rabbit antiserum increased, the precipitation patterns simulated the 3 (9.3 per cent), 2 (21.0 per cent), and 1 (61.5 per cent)-plus reactions frequently observed with human sera giving positive gel diffusion test reactions (1).

Therefore, since the various degrees of positive test reactions could be duplicated by diluting the 4-plus rabbit antiserum in tests in which all other reagents were held constant, the second assumption as given in the Introduction appears to be valid and, therefore, justifies the method used for recording degrees of positive reactions.

Since it did appear that sera from most humans

naturally antigenized through infection with *Mycobacterium tuberculosis* contained small amounts of specific antibody, a means was sought for increasing the magnitude of these positive reactions. The method evolved consisted of removing and discarding the serum in the gel diffusion test at regular intervals during incubation and replacing it with unused serum. Theoretically, as will be pointed out in the Discussion, this technique should result in an intensification of the precipitate bands.

Six twofold dilutions of rabbit antiserum were prepared, and each serum dilution was used in a gel diffusion test. All tubes were observed at each twenty-four-hour interval, and fresh serum dilution was used to replace the initial serum in each test. At the end of five days, serum had been replaced four times in all tests of each dilution of serum. The number of bands observed in tests of each dilution on each day of incubation is given in table 1. This experiment was performed three times with both H37Rv and H37Ra antigen and respective antibody with similar results.

TABLE 1

RESULTS OF ANTISERUM REPLACEMENT TESTS

Antiserum Dilution	H37Ra System					H37Rv System				
	Number of Bands per Incubation Day					Number of Bands per Incubation Day				
	1	2	3	4	5	1	2	3	4	5
Undiluted	5	6	3*	*	*	4	6	*	*	*
1:2	5	6	3*	*	*	3	4	6	6	*
1:4	3	6	6	6	6	2	3	5	6	6
1:8	0	2	4	6	6	0	2	4	6	6
1:16	0	0	3	3	5	0	0	1	2	4
1:32	0	0	1	2	2	0	0	0	1	2
1:64	0	0	0	0	1	0	0	0	0	1

* Due to continuous accumulation of antigen-antibody complexes, these bands became very heavy, coalesced, and could not be counted.

Considering the H37Ra reactions noted in table 1, it is apparent that only after forty-eight hours of incubation both the 1:2 dilution and the undiluted rabbit serum showed the maximum of 6 clearly discernible bands of precipitate. At seventy-two hours, however, the precipitate in the undiluted serum tubes had become so voluminous that the separate bands could not be counted. The maximal reaction was seen in the 1:4 dilution tubes at this time. At ninety-six hours the maximal reaction was observed in the 1:8 as well as in the 1:4 dilution tubes. This pattern of uniform progression of maximal reaction through a series of serially diluted specimens of serum was similarly observed with the H37Rv system. Figure 2 is a photograph of the H37Ra antigen and antibody reactions at the third day of incubation. From this photograph, the gradual decrease in both number and intensity of precipitate bands can be seen as the serum is diluted. The third tube (1:8 dilution) shows the maximal countable number of bands at this time.

Since the serum-replacement technique could produce a progression of maximal reaction through a series of diluted sera as shown above, it was decided to test this technique as a possible means for increasing sensitivity of the gel diffusion test for detecting antibody in low-titer human sera.

Twenty-one pooled human serum specimens were prepared, each specimen containing serum from 15 to 28 individual patients. These pooled serum samples were set up in gel diffusion tests as follows: Another triplicate set of 21 tubes each was prepared in the routine manner, using a 2:1

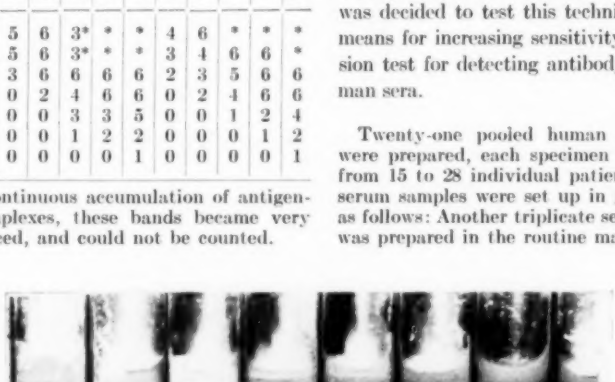

FIG. 2. Precipitation reactions after three days of incubation in 7 gel diffusion tubes when a twofold dilution of antibody was employed.

TABLE 2

RESULTS* OF GEL DIFFUSION SERUM REPLACEMENT TESTS USING POOLED HUMAN-SERUM SPECIMENS

Serum Number	I†	II‡	III§
1	0	3+	2+
2	1+	3+	2+
3	1+	2+	2+
4	0	3+	1+
5	0	1+	1+
6	0	2+	0
7	0	2+	0
8	0	3+	2+
9	1+	3+	2+
10	1+	2+	1+
11	1+	3+	2+
12	0	0	0
13	0	1+	0
14	1+	2+	1+
15	0	3+	1+
16	0	2+	1+
17	1+	3+	2+
18	0	2+	1+
19	1+	3+	2+
20	1+	3+	2+
21	1+	2+	1+

* Given in terms of 1- to 4-plus reactions.

† Control series. Serum was not replaced in this set of tests.

‡ Set up identically to I, but serum was replaced in each tube daily for 5 days.

§ Set up as I and II, except the center column was increased to 2.0 cm. Serum was changed daily for 5 days.

ratio of antigen to agar in the base layer, and served as a control set. The average of the reactions observed in the triplicate tests in this set is given in column 1 of table 2. Three other sets of tubes were similarly prepared. The center column in both the first and second sets was 1.0 cm. long. In these latter 3 sets of tubes, however, fresh serum was used to replace the serum layer at twenty-four-hour intervals. The average results from the triplicate tests in this second set are given in column 2 of table 2. Column 3 in table 2 represents the average of the results obtained from a third triplicate set of tubes. The tests in this third set were performed with a 2.0 cm. center diffusion column rather than the 1.0 cm. column as used in sets 1 and 2, and the serum in this set was also replaced at twenty-four-hour intervals.

When the three columns of figures in table 2 are compared, it becomes evident that serum replacement did effect an increase in magnitude of reaction. This was found to be more evident in the second set of tubes, in which the diffusion column was maintained at 1.0 cm. and serum was replaced at twenty-four-hour intervals, than for the third set of serum replacement tubes, in which the length of the center column was increased to 2.0 cm.

Since the only variable between the tests which produced the results given in column 2 and the tests responsible for those in column 3 was the increased length of the center column, it would seem that the more marked reactions seen in column 2 were due to the shorter reaction column. This observation is consistent with that of Wilson (9) who found that the shorter the distance of diffusion the greater was the sensitivity of the test.

It is noteworthy, however, that, even with the increase in length of the center column, final test readings given in column 3 exceed those in column 1, indicating that the mechanism of serum replacement would be an effective simple method to use for enhancing gel diffusion reactions with serum of low antibody titer.

The third purpose of this report is to present a technique which could be used to determine whether the effectiveness of two antigen preparations prepared at different times would react in a comparable way in gel diffusion tests. Initially, newly prepared and old antigen preparations were tested against a reference rabbit antiserum, and the reactions obtained with both were compared. From such visual comparisons it was possible to determine approximately if the two aliquots of antigen were of nearly equal precipitating potency. However, since antigen excess effects were much more evident in testing low-antibody titer serum as compared with high-antibody serum, a more sensitive means of comparing the precipitating activity of two batches of antigen is needed. This was accomplished as follows.

Each batch of antigen was serially diluted, and each dilution was used as the reagent to be placed over the second agar column in the tubes. The base agar column of each test tube consisted of a 4:1 ratio of rabbit antiserum to 2 per cent agar. Care was taken to maintain uniformity of height in all agar columns. These tests were incubated, the number of bands per tube per day recorded, and the antigen dilutions were replaced in exactly the same way as in the serum replacement experiment described above.

It may be seen in table 3 that several antigen effects could be observed in such a series of tests. It was possible to follow the progression of maxi-

TABLE 3

RESULTS OF ANTIGEN REPLACEMENT TESTS

Antigen Dilution	H37Ra System* Number of Bands per Incubation Day					H37Rv System* Number of Bands per Incubation Day				
	1	2	3	4	5	1	2	3	4	5
Undiluted	5	4	4	3	2	5	6	6	4	2
1:2	2	6	6	5	4	3	6	6	6	4
1:4	1	6	6	5	5	2	5	6	6	5
1:8	1	4	6	6	5	0	4	5	6	6
1:16	0	3	5	6	6	0	3	4	5	6
1:32	0	2	4	4	5	0	2	3	4	5
1:64	0	1	3	4	4	0	1	1	3	3
1:128	0	0	1	2	2	0	0	0	1	2
1:256	0	0	0	0	1	0	0	0	1	1
1:512	0	0	0	0	0	0	0	0	0	1

* A 4:1 ratio of immune rabbit serum to agar was used as a diffusion source for antibody in all tubes.

mal and minimal antigen-antibody reactions through the series of diluted antigens from the first to the fifth day of incubation. Furthermore, because dissolution of some precipitate by excess antigen was observed, it was also possible to follow antigen excess levels in a similar manner.

If the results of the H37Ra series are considered (table 3), it would appear that excess antigen in the undiluted antigen tests inhibits maximal reaction in less than twenty-four hours; therefore, the maximum of 6 precipitate rings was never seen in tests using undiluted antigen. When antigen was diluted 1:2, the observed excess antigen effect was decreased so that the last maximal reaction was recorded on the third day and after two replacements of the 1:2 antigen dilution. With more time and after replacing the antigen a third time, sufficient excess antigen apparently diffused into the second layer and effected dissolution of two of the six antigen-antibody complexes. The time of appearance, as well as the duration of maximal reaction, was nearly comparable with the 1:2 dilution in the 1:4 dilution system, although perhaps slightly slower in rate as suggested by the difference of 2 bands in the 1:2 dilution tube and a single band in the 1:4 dilution tubes at twenty-four hours. In the other dilutions, greater than 1:4, it may be seen that the optimal reactions appeared successively later day by day. The experiment was terminated on the fifth day, at which time a single precipitate band was apparent in the tubes prepared with an antigen dilution of 1:256. This reaction was comparable in appearance with the single band observed in the 1:4 antigen dilution tube at twenty-four hours of incubation. In general, the pattern of reactions observed with the H37Rv series was comparable with the H37Ra tests.

Therefore, since these antigen dilution experiments demonstrated that a direct relationship existed between antigen concentration and the degree of positive reaction encountered in gel diffusion systems, such a simple titration procedure would comprise a useful means for comparing precipitating potency possessed by separate preparations of antigen. The same principles involved in the optimal proportions tests, as used in antigen-antibody flocculation procedures, govern this type of titration.

DISCUSSION

The recent evaluation study of a gel double-diffusion precipitation technique demonstrated that specific antibody was frequently present in serum from tuberculous patients (1). When the quantitative aspects of the gel double-diffusion reactions were considered, it was noted that this antibody was most often present in relatively small amounts. The experiment in which dilutions of 4-plus positive immune rabbit serum were used to duplicate the various degrees of positive reactions encountered in testing positive human sera has experimentally confirmed the validity of this observation.

Gel diffusion techniques apparently are of greater sensitivity in detecting low concentrations of antibody than are the ring precipitation tests. Recently, Wilson (9) has quantitatively measured the extent of this greater sensitivity of gel double-diffusion systems. In addition, he has also outlined some of the mechanical factors which regulate this sensitivity. Wilson determined that, under certain well-defined conditions, the magnitude of the difference in sensitivity of the gel diffusion test over the ring test is nearly fourfold. This greater sensitivity possibly explains why the gel double-diffusion precipitation technique appears to be more satisfactory for the detection of antibody in human sera to mycobacterial antigens. A precipitating serologic technique, in order to be diagnostically useful, must produce sufficient antigen-antibody complex to be visible. There is, therefore, a minimal quantity of

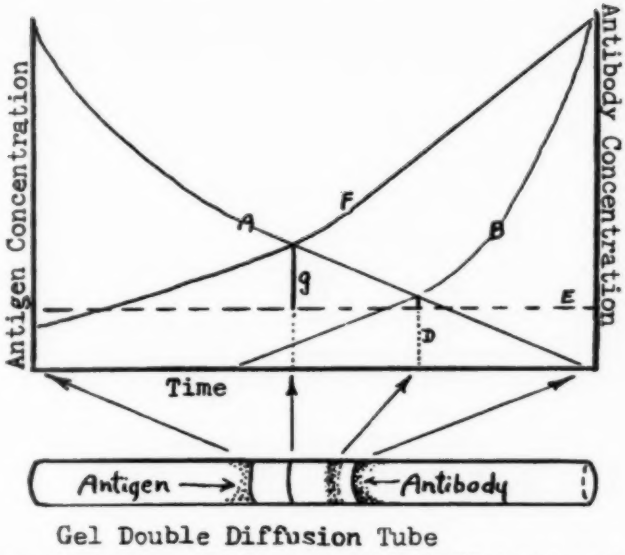

FIG. 3. A diagrammatic illustration of the dynamics of gel double-diffusion tubes and of how precipitation develops.

antibody which will produce a visible reaction with the antigen preparation employed. Antibody which appears in serum in concentrations below this minimum may still combine with antigen but will not produce a visible reaction. In effect, a threshold of visibility in any antigen-antibody precipitation system exists that is primarily governed by the volume of antibody in the precipitate.

This generalization can be related to the double diffusion precipitation by means of the schematic illustration represented by figure 3. The parabolas *F* and *B* represent the concentration gradients of two different antibodies, respectively, as they diffuse through the agar medium. The parabola *A* represents a similar concentration gradient of two different antigens specific for antibodies *F* and *B*. Although its actual occurrence would be unlikely, the assumption can be made that these two antigens are of equal diffusibility and are present in equal concentrations. This assumption simplified the diagram and permits the single parabola to represent two different antigens. The broken line *E* represents the threshold of visibility governed by antibody concentration as discussed above. Below this threshold of visibility, even though optimal proportions of complete antibody and antigen may be achieved, low concentrations may not permit their visible detection. Under this condition a serologic test for specific antibody would be negative due to nonvisualization of formed antigen-antibody complex. If the equivalence ratio favoring visible precipitation occurs just above the visibility threshold, the precipitate band may appear faint and rather diffuse. On the other hand, if the intersection of antigen and antibody concentration parabolas occur well above the visibility threshold, the resulting precipitate will appear as a dense sharply defined disc.

It is important to recognize this qualitative difference in precipitate bands, as well as to have some understanding of the mechanism of their formation. The faint diffuse bands which appear close to the antiserum agar meniscus in the gel double-diffusion tubes are easily confused with faint nonspecific precipitations which occasionally develop due to chemical reactions between badly hemolyzed, lipemic, contaminated, or otherwise altered serum and impurities in the agar. This creates an area for possible error, since a very narrow line exists between negative and weakly positive gel diffusion precipitation reactions. It is very easy to obtain false positive readings in

nontuberculous serum due to such artifactual clouding of agar; or, on the other hand, if these faint precipitates are disregarded, to obtain a high number of falsely negative results. This possibly accounts for some of the erroneous serologic findings reported in the evaluation study (1).

In order to widen the margin between positive and negative reactions, there are several possibilities. The more obvious would be to intensify all specific precipitation reactions by using serum with a more concentrated antibody content. Such concentration of serum is possible through lyophilization or evaporation in order to reduce overall serum volume, but this would be laborious and impractical for a clinical laboratory. It can be more easily accomplished, however, through the serum-replacement technique described in this paper, since more antibody is being constantly supplied to the system. Without this procedure, in which diffusion of antibody from the overlying antiserum into the reaction column progresses with time, the concentration of antibody progressively builds up in the center reaction column and decreases in the overlying serum reservoir. When serum is replaced at regular intervals during incubation, however, the reduction of concentration of diffusible antibody in the serum reservoir is considerably retarded. This retardation of antibody depletion from the reservoir apparently affects formation of the antigen-antibody complex in the same manner as would the use of more concentrated antiserum. This is a simple practical method of intensifying gel diffusion precipitation reactions when using antiserum of low antibody strength. Such an intensification of precipitation reactions automatically widens the gap between positive and negative tests and would constitute one means of increasing the sensitivity of the gel double-diffusion test for tuberculosis.

From an earlier investigation of mycobacterial antigen relationships (8), it was determined that the *hominis* strains possess a minimum of 6 different antigenic substances. All attempts of these investigators to isolate these substances in purified form have met with failure to date. Therefore, the concentrated unheated culture filtrate antigen mixtures remain to date the most effective antigen preparation for use in gel diffusion precipitation tests. It is apparent that, if these crude antigen mixtures are going to be used in a gel double-diffusion test for tuberculosis, some means of determining uniformity in reactivity from one preparation to another would be necessary. The serial antigen dilution-replacement method proposed in this paper can function in this capacity.

In actual performance of the tests, antigens of uniform reactivity standardized in the manner described herein should be used. This would minimize error due to variation of antigen reactivity from one batch to the next. In order to minimize error in reading tests due to precipitations occurring near the visibility threshold, it might be well to observe reactions daily, record all definitely positive tests appropriately as to degree of reaction and day observed, then replace serum on all negative, very weakly positive or doubtful tests. If this were performed daily, more of the low antibody titer serum would be detected and the sensitivity of the test would be appreciably improved.

Summary

The present investigation had a threefold purpose. First, through dilution of rabbit antiserum which contained a high concentration of precipitating antibodies to mycobacterial antigens, it was possible to simulate all degrees of reaction observed between human serum and culture filtrate antigens in the gel diffusion test for tuberculosis. Projecting this finding to interpretation of such test results on 1,097 serum specimens from hospital patients which are reported elsewhere (1), the contention was verified that antibodies to mycobacterial antigens found in the serum as a result of tuberculous infection are most often present in relatively low concentration.

A second purpose was achieved by the demonstration of precipitate band intensification through a technique of replacing serum in diffusion tests at regular intervals during incubation. This intensification effect was observed with both immune rabbit serum and pooled samples of serum obtained from persons with active tuberculosis. This technique may be applied to render the gel diffusion test for tuberculosis more sensitive.

Finally, because there was a need for a reliable, sensitive means of comparing the ability of two aliquots of an antigen mixture to precipitate antibody equally, an antigen titration for the gel diffusion test has been devised.

Sumario

Nuevos Estudios de las Pruebas de Difusión del Gel en la Tuberculosis. I. Métodos para la Estandardizacion del Antigeno y el Descubrimiento de Pequeñas Cantidades de Anticuerpo

La investigación actual tenía un triple fin. Primero, por medio de la dilución del antisuero de conejo que contenía una elevada concentración de anticuerpos precipitantes para los antígenos micobacterianos, fué posible simular todos los grados de las reacciones observadas entre el suero humano y los antígenos de filtrados culturales en la prueba de difusión del gel en la tuberculosis. Proyectando este hallazgo a la interpretación de los resultados de la prueba en 1,097 ejemplares de suero, procedentes de enfermos hospitalizados, que se han presentado en otra parte (1), se comprobó la alegación de que los anticuerpos para los antígenos micobacterianos descubiertos en el suero a consecuencia de la infección tuberculosa alcanzan más a menudo una concentración relativamente baja.

Se logró otro propósito por el descubrimiento de la intensificación de una franja de precipitado por medio de una técnica de substitución del suero en las pruebas de difusión a plazos regulares durante la incubación. Este efecto intensificante se observó tanto con el inmunisuero de conejo como con muestras combinadas de suero obtenido de personas que tenían tuberculosis activa. Puede aplicarse esta técnica para sensibilizar más la prueba de difusión del gel para la tuberculosis.

Tercero, por haber necesidad de un medio sensible y fidedigno para comparar la capacidad de dos partes alicuotas de una mezcla de antígenos para precipitar igualmente el anticuerpo, se ha elaborado una titulación del antígeno para la prueba de difusión del gel.

Resume

Nouvelles études sur les tests de diffusion dans le gel dans la tuberculose. I. Méthodes de standardisation de l'antigène et de détection de petites quantités d'anticorps

Cette étude avait un triple but. Tout d'abord, en diluant de l'antisérum de lapin contenant une concentration élevée d'anticorps précipitants aux antigènes mycobactériens, il a été possible de simuler tous les degrés de réactions observées entre le sérum humain et la culture de filtrat d'antigènes dans le test de diffusion dans le gel dans la tuberculose. En appliquant cette observation à l'interprétation des résultats de tests obtenus sur 1097 spécimens de sérum de malades hospitalisés, dont les cas ont été rapportés ailleurs (1), les auteurs ont pu vérifier la théorie suivant laquelle, au cours d'une infection tuberculeuse, les anticorps mycobactériens circulant dans le sérum s'y trouvent en concentration relativement peu élevée.

Le deuxième but a été atteint en démontrant l'intensification de la zone de précipitation par le remplacement du sérum dans les tests de diffusion à intervalles réguliers pendant l'incubation. Cet effet d'intensification a été observé avec le sérum de lapin immunisé d'une part, et un mélange d'échantillons de sérum de malades présentant une tuberculose en activité, d'autre part. Cette technique peut être employée pour rendre plus sensible le test de diffusion dans le gel dans la tuberculose.

Troisièmement, comme il a été nécessaire de trouver un procédé sensible et sûr permettant de comparer le pouvoir des deux parties de mélange antigénique de précipiter également des anticorps, un titrage antigénique pour le test de diffusion dans le gel a été trouvé.

REFERENCES

(1) Parlett, R. C., and Youmans, G. P.: An evaluation of the specificity and sensitivity of a gel double-diffusion test for tuberculosis, Am. Rev. Resp. Dis., 1959, *80*, 153.

(2) Ouchterlony, O.: Antigen antibody reactions in gels: IV. Types of reactions in coordinated systems of diffusion, Acta path. et microbiol. scandinav., 1953, *32*, 231.

(3) Becker, E. L., and Munoz, J.: Antigen-antibody reactions in agar: I. Complexity of antigen-antibody systems as demonstrated by a serum-agar technique, J. Immunol., 1950, *65*, 47.

(4) Becker, E. L.: Antigen-antibody reactions in gels, Fed. Proc., 1953, *12*, 717.

(5) Becker, E. L., Munoz, J., Lapresle, C., and LeBeau, L. J.: Antigen-antibody reactions in agar: II. Elementary theory and determination of diffusion coefficients of antigens, J. Immunol., 1951, *67*, 501.

(6) Oakley, C. L., and Fulthorpe, A. J.: Antigenic analysis by diffusion, J. Path. & Bact., 1953, *65*, 49.

(7) Parlett, R. C., Youmans, G. P., Rehr, C., and Lester, W.: The detection of antibodies in the serum of tuberculous patients by an agar double-diffusion precipitation technique, Am. Rev. Tuberc., 1958, 77, 462.

(8) Parlett, R. C., and Youmans, G. P.: Antigenic relationships between ninety-eight strains of mycobacteria using gel-diffusion precipitation techniques, Am. Rev. Tuberc., 1958, 77, 450.

(9) Wilson, M. W.: The agar diffusion precipitin technique: A comparison of the simple and double diffusion methods, J. Immunol., 1958, *81*, 317.

Additional Bibliography

Oudin, J.: L'analyse immuno-chimique qualitative: Méthode par diffusion des antigènes au sein de l'immunsérum précipitant gélosé, Ann. Inst. Pasteur, 1948, *75*, 30.

Oudin, J.: Methods in Medical Research, Vol. 5, Year Book Publishers, Inc., Chicago, Illinois, 1952, pp. 360–375.

Wilson, M. W., and Pringle, B. H.: Interpretation of the Ouchterlony precipitin test, J. Immunol., 1955, *75*, 460.

Halbert, S. P., Swick, L., and Sonn, C.: The use of precipitin analysis in agar for the study of human streptococcal infections: II. Ouchterlony and Oakley technique, J. Exper. Med., 1955, *101*, 557.

Schiott, C. R.: Preliminary studies on antigen-antibody reactions in gels according to Ouchterlony's method, Acta path. et microbiol. scandinav., 1953, *32*, 251.

Case Report

ALVEOLAR-CAPILLARY BLOCK DUE TO LEUKEMIC INFILTRATION OF THE LUNG[1]

ROBERT A. GREEN,[2] NORMAN J. NICHOLS, AND EDWARD J. KING

(Received for publication April 20, 1959)

Introduction

The lung is not ordinarily considered a common site of involvement in leukemia. Although autopsy studies have revealed a modest incidence of histologic abnormalities in the lungs, clinical manifestations of pulmonary disease have rarely been reported.

In 1955, Nathan and Sanders (1) described a patient with acute myelogenous leukemia in whom pulmonary insufficiency occurred that appeared to be due to leukemic infiltration. The patient's symptoms and roentgenographic abnormalities responded to therapy with amethopterin. On clinical grounds the writers characterized the presenting syndrome as one of alveolar-capillary block. It had not previously been suggested that this syndrome could be caused by leukemic involvement of the lung.

It is the purpose in the present study to report the clinical, physiologic, and post-mortem findings in a patient with chronic lymphatic leukemia who developed alveolar-capillary block.

Case Report

A 45-year-old white salesman was admitted to the Veterans Administration Hospital, Bronx, New York, on November 16, 1956, complaining of shortness of breath and chest pain.

In April, 1956, a routine chest roentgenogram revealed abnormalities which were interpreted as "viral pneumonia." His only symptom at this time was mild chronic cough productive of mucoid sputum. Despite a course of therapy with tetracycline, the roentgenographic abnormalities persisted, and the patient was admitted to another hospital. Various studies, including bronchoscopy, failed to establish a diagnosis. Following bronchoscopy, blood-streaked sputum and discomfort in the xiphoid region developed. These symptoms recurred intermittently thereafter. In October, 1956, he noted the abrupt onset of severe progressive dyspnea which quickly became incapacitating. There was no orthopnea, paroxysmal nocturnal dyspnea, edema, or previous history of pulmonary or cardiac disease. One month later the patient was admitted to this hospital.

Physical examination on admission revealed a well-developed, well-nourished male with a florid appearance. He complained of severe dyspnea at rest. The temperature was normal, pulse rate 88, respirations 32 per minute, and blood pressure 120 systolic, 78 diastolic in mm. of mercury. Firm discrete lymph nodes, 0.5 to 1.5 cm. in size, were found in the suboccipital, cervical, and axillary regions bilaterally. Matted nodes were noted in the inguinal regions. The respiratory excursions were good. The percussion note over the lung fields was normal, and there was no abnormality of the breath sounds. Crepitant rales were heard over the right lower hemithorax. The heart was normal. A firm, smooth, nontender liver edge was felt three finger-breadths below the right costal margin. A firm spleen tip was palpated four finger-breadths below the left costal margin.

On admission the hemoglobin was 16.3 gm. per 100 ml.; it never fell lower than 13.0 gm. The leukocyte count was 130,000, and the differential count revealed 14 per cent segmented neutrophils, 5 per cent band forms, 2 per cent metamyelocytes, 69 per cent lymphocytes, and 10 per cent monocytes. Several normoblasts were also noted. The lymphoctes appeared mature. The platelet count was 172,000. The urine gave a three-plus test for albumin, and was otherwise normal. An electrocardiogram was normal. Chest roentgenograms (figure 2) revealed a normal heart and no definite evidence of hilar or mediastinal adenopathy. Diffuse reticular infiltrates were noted throughout both lung fields. A roentgenogram taken elsewhere two months previously (figure 1) showed that the process was limited to the left lower lung field at that time.

Dyspnea rapidly increased and the patient was soon severely distressed even at complete rest. Bone-marrow aspirations were performed on two occasions. Each time the marrow was cellular. Approximately 50 per cent of the cells were adult lymphocytes, but occasional earlier forms were noted. The myeloid series appeared normal. The erythroid elements were adequate in number. Some of the normoblasts showed fragmented nuclei. Megakaryocytes were somewhat reduced. The bone-marrow and peripheral blood findings were compatible with either chronic lymphatic

[1] From the Chest Section of the Medical Service and the Cardiopulmonary Laboratory, Veterans Administration Hospital, Bronx, New York.

[2] Present address: Veterans Administration Hospital, Ann Arbor, Michigan.

leukemia or a leukemoid reaction, and some difference of opinion existed as to which was present. On the eighth hospital day pruritus and marked jaundice were noted. The total serum bilirubin was 16.3 mg. per 100 ml., of which 8.5 mg. per 100 ml. was direct reacting and 7.8 mg. per 100 ml. was indirect. Other chemical tests revealed that the cephalin flocculation was 2-plus; thymol turbidity, 12.0 units; alkaline phosphatase, 25.9 units (Shinowara); serum albumin, 3.3 gm. per 100 ml.; serum globulin 2.6 gm. per 100 ml.; and the prothrombin content, 55 per cent. The blood urea nitrogen was 25 mg. per 100 ml. Bile was present in the urine.

On the tenth hospital day, mental confusion and a flapping tremor developed. Arterial blood studies revealed an oxygen capacity of 19 volumes per cent, oxygen content of 14.7 volumes per cent, and saturation of 77.5 per cent. The partial pressure of oxygen was markedly reduced to 42 mm. of mercury. The arterial carbon dioxide tension was reduced to 26 mm. of mercury. The carbon dioxide content was normal (28 mEq. per liter). The blood pH was at the upper limit of normal at 7.46. These figures were interpreted as revealing marked impairment of oxygen diffusion. It was believed that a neoplastic infiltrative process had involved both the lungs and the liver, thus accounting for the difficulty in oxygen transport and the cholemic state.

The patient was treated with antimicrobials, oxygen, sodium glutamate, and steroids. Jaundice deepened, ascites developed, and the mental status deteriorated further. On the twelfth hospital day radiation therapy to the liver was begun. Doses of 75 roentgens were given on the twelfth and 100 roentgens on the thirteenth days. However, on the thirteenth hospital day marked oliguria was noted. Urinary output was less than 100 ml. The blood urea nitrogen rose to 91 mg. per 100 ml., and the uric acid was 16.9 mg. per 100 ml. On the fifteenth hospital day the patient died, deeply jaundiced and cyanotic.

At autopsy, cyanosis, intense jaundice, and petechiae were noted in the skin and in most of the tissues. The liver was considerably enlarged, weighing 3,500 gm. On cut surface, hepatic lobular architecture was strikingly accentuated by congestion of the central areas and replacement of the remainder of the lobules by tan tissue. This change occurred diffusely throughout the liver. The spleen weighed 1,350 gm. Most of the lymph node groups contained enlarged nodes. The marrow was deep red in color. Microscopic examination of the liver revealed a remarkable degree of cellular lymphocytic infiltration throughout the entire hepatic parenchyma. The change was most marked in the portal areas of each lobule. The spleen and lymph nodes were also diffusely replaced by relatively mature lymphocytic cells. The fixed sections of marrow demonstrated increased cellularity. Myeloid and erythroid elements as well as megakaryocytes were easily identified, although in somewhat diminished numbers. Most of the cellularity was due to diffuse infiltration with early lymphocytes, similar to those seen in the pre-mortem marrow specimens. The cardiovascular, gastrointestinal, and genitourinary systems showed no major gross or microscopic alterations. Although the bone marrow alone was consistent with either leukemia or a severe lymphatic leukemoid reaction, the sections of lymph node, liver, and spleen established the diagnosis of chronic lymphatic leukemia.

FIG. 1 (Left). Roentgenogram, postero-anterior view, two months prior to admission. Infiltration is limited to the left lower lung field.

FIG. 2 (Right). Roentgenogram, postero-anterior view, on admission. Diffuse bilateral infiltration is present.

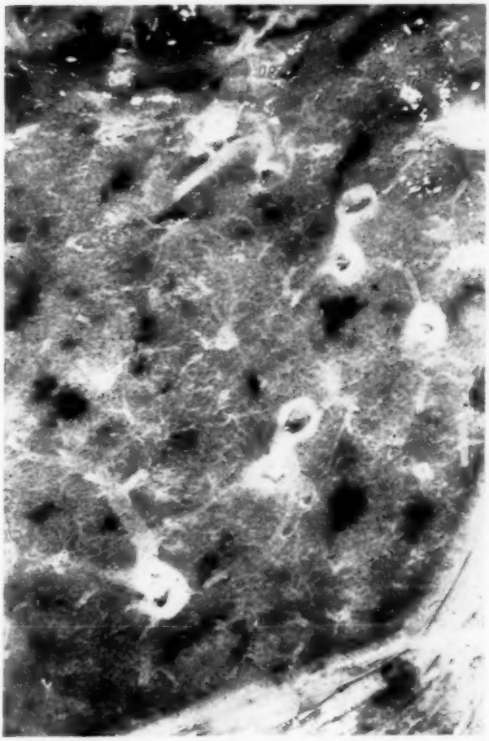

FIG. 3. Left upper lobe. Gross appearance of cut surface at autopsy.

Grossly the lungs were striking. Each weighed approximately 800 gm. Fibrous adhesions covered the pleura over the left lower lobe. Crepitation was generally reduced. The cut surface of both lungs was pale and somewhat firmer than normal. On close inspection delicate weblike tissue extended throughout the lungs and appeared to outline the lobules and smaller bronchiolar structures, imparting an over-all fine microcystic appearance to the lungs (figure 3). This change was most easily discernible in the upper lobes and in the right middle lobe. The lower lobes appeared more congested and diffusely consolidated. The trachea, bronchi, and pulmonary vessels were essentially normal.

Microscopic examination revealed that all areas of both lungs were diffusely infiltrated with lymphocytes. The process was not focal: although the degree of involvement varied from area to area, no section of lung examined microscopically was free of leukemic infiltration. Coalescence of lesions was noted in the perivascular and peribronchial areas, and often the bronchial walls themselves were diffusely infiltrated. Small adventitial lymphatic vessels in the walls of the bronchi and arteries were often choked with leukemic cells. The blood vessels contained many lymphocytes, comparable to the peripheral lymphocytosis noted during life. The pleura and subpleural areas were heavily invaded, and from here the process extended into the lungs along interlobular septa (figure 4).

In the upper lobes, the parenchyma was severely involved adjacent to the pleura and peribronchial areas; here, the diffuse interstitial involvement was quite fine (figure 5), with resultant preservation and even accentuation of pulmonary architecture (figure 6). The leukemic cells widened and thickened the alveolar septa, and were definitely located in the interstitial spaces between the capillary and alveolar lining cells. This was most striking in the areas of heaviest involvement which were in the lower lobes, in which the architecture of the lung was no longer recognizable because of the extreme degree of cellular infiltration (figure 7). In the left lower lobe a considerable amount of interstitial fibrosis was associated with the cellular deposition (figure 8), and very rarely the fibrosis predominated, with minimal cellular infiltrate (figure 9). Pleural and subpleural fibrosis

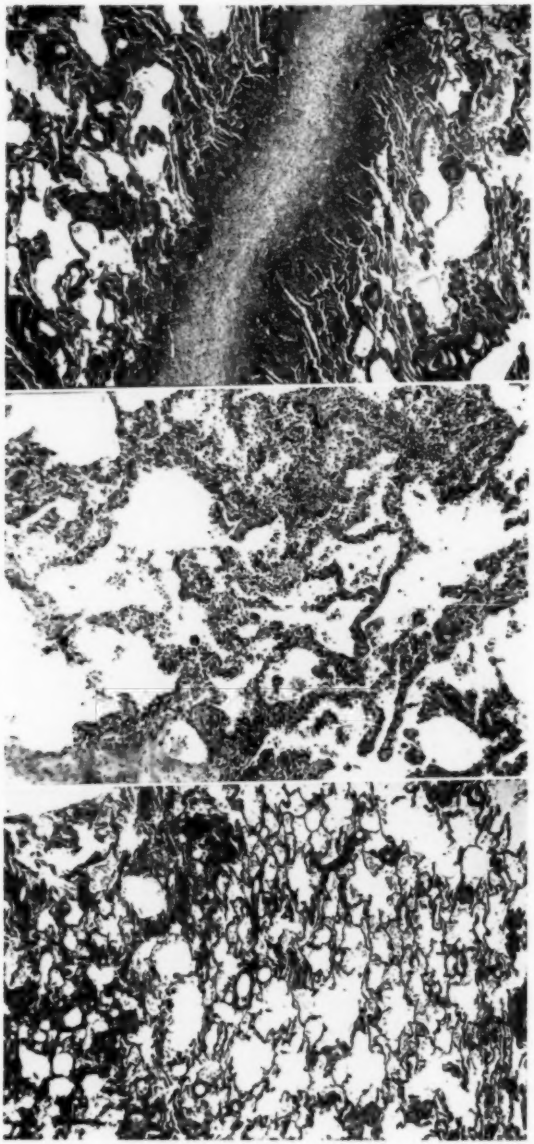

FIG. 4 (Top). Left lung. Infiltration of interlobar fissure. (×27)
FIG. 5 (Center). Left upper lobe. Fine interstitial infiltration. (×98)
FIG. 6 (Bottom). Right upper lobe. Fine interstitial infiltration. Note perivascular foci. (×27)

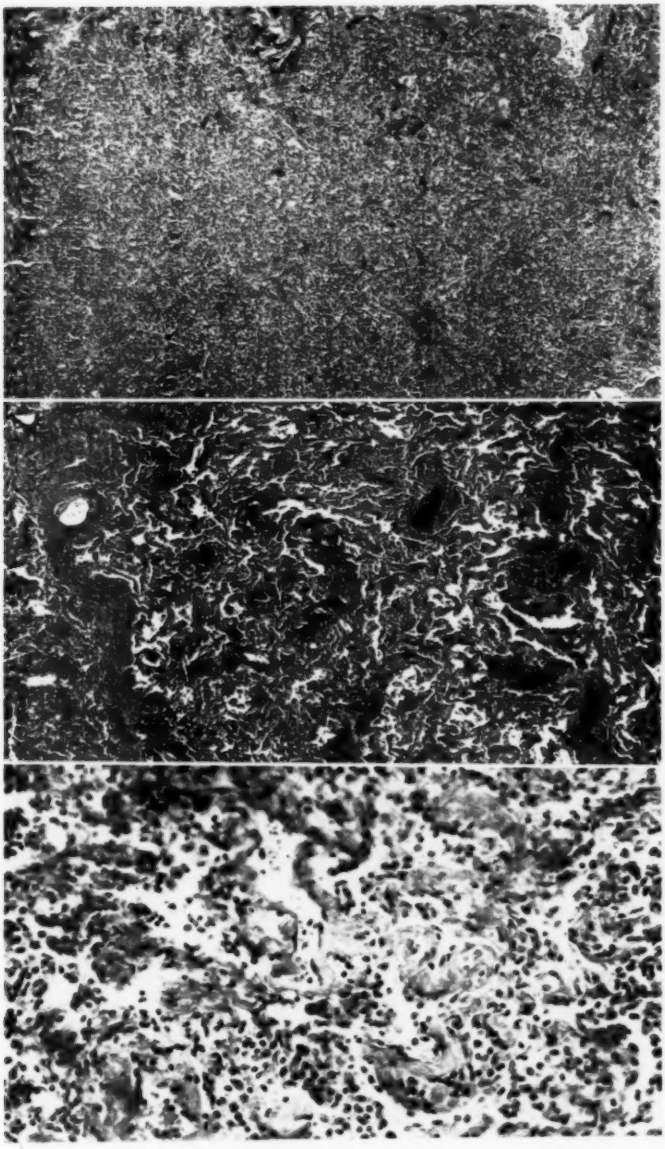

FIG. 7 (Top). Left lower lobe. Heavy infiltration. (×30)
FIG. 8 (Center). Left lower lobe. Interstitial infiltration and fibrosis. (×30)
FIG. 9 (Bottom). Left lower lobe. Interstitial fibrosis. (×288)

was also seen. Minor scattered areas of fibrosis were noted elsewhere in the lungs.

The significant autopsy diagnoses were: chronic lymphatic leukemia; massive leukemic infiltration of lungs and liver; moderate leukemic infiltration of spleen, lymph nodes, and bone marrow; minor leukemic infiltrates of skin, kidneys, bladder, and gall bladder; bile nephrosis.

Discussion

Alveolar-capillary block, the functional pulmonary abnormality from which the patient suffered, was first clearly described by Austrian and associates (2) in 1951, although a disturbance, in which the primary abnormality is an altered permeability of the alveolar-capillary membrane, had been proposed in the German literature as early as 1922. In 1935, Hamman and Rich (3) published their initial report of a clinical and pathologic syndrome in which extensive thickening of the alveolar-capillary membrane, due to fibrosis of unknown etiology, was found. Baldwin and associates (4) reported 14 cases of various chronic pulmonary diseases, including pulmonary scleroderma, lymphangitic carcinomatosis, and pulmonary granulomatosis and fibrosis of unknown etiology, in which the primary functional disturbance was either a veno-arterial shunt or impairment of the diffusion of respiratory gases across the alveolar-capillary interface. Austrian and co-workers (2), aided by refinements in pulmonary physiologic methodology, proved that the difficulty was due to impaired diffusion rather than to abnormal shunting. The pulmonary function studies in this syndrome are characterized by reduced lung volumes, maintenance of relatively large maximal breathing capacity, hyperventilation at rest and with exercise, slight to marked reduction in arterial oxygen tension and saturation at rest, and further reduction with exercise, normal alveolar oxygen tension, reduced diffusing capacity, and normal or low arterial carbon dioxide tension. The series of Austrian and associates (2) included cases of beryllium granulomatosis and sarcoidosis. Other suggested etiologies for the syndrome include radiation pneumonitis and fibrosis (5), miliary tuberculosis, sulfur dioxide poisoning, silo-filler's disease, and hexamethonium sensitivity. The pathology that these varied diseases have in common is extreme thickening of the alveolar-capillary membrane.

The characteristic clinical picture of the alveolar-capillary block syndrome was exhibited by the patient of the present case. Severe unremitting dyspnea, marked hyperventilation, and cyanosis were present, in the absence of neuromuscular deficiency, obstruction to airflow, or heart failure. The patient's pre-terminal state precluded pulmonary function studies other than analysis of the arterial gases. The low arterial oxygen tension (42 mm. of mercury) and saturation (77.5 per cent) associated with a marked reduction in arterial carbon dioxide tension (26 mm. of mercury) in this clinical setting indicated impaired diffusion across the alveolar-capillary membrane. The autopsy findings confirmed this diagnosis, as the major site of pathology in the lung was in the interstitial tissue between the capillary lumina and alveolar spaces. Although some interstitial fibrosis was found, this was of minor degree and occurred only in scattered areas; it is apparent that the massive infiltration by leukemic cells was mainly responsible for the physiologic abnormality.

Summary

The case of a patient with chronic lymphatic leukemia is presented in whom massive infiltration of the lung by leukemic cells caused fatal pulmonary insufficiency of the alveolar-capillary block type.

Sumario

Bloqueo Alvéolo-Capilar Debido a Infiltración Leucémica del Pulmón

Preséntase el caso de un enfermo de leucemia linfática crónica en el cual la infiltración masiva del pulmón por células leucémicas ocasionó insuficiencia pulmonar letal de la forma de bloqueo alvéolo-capilar.

Resume

Blocage alvéolo-capillaire dû à l'infiltration leucémique du poumon

L'auteur rapporte le cas d'un malade atteint de leucémie lymphatique chronique, chez qui l'infiltration massive du poumon par des cellules leucémiques a provoqué une insuffisance pulmonaire mortelle, caractéristique de blocage alvéolo-capillaire.

REFERENCES

(1) Nathan, D. J., and Sanders, M.: Manifestations of acute leukemia in the parenchyma of the lung, New England J. Med., 1955, *252*, 797.

(2) Austrian, R., McClement, J. H., Renzetti, A. D., Jr., Donald, K. W., Riley, R. L., and Cournand, A.: Clinical and physiologic features of some types of pulmonary diseases with impairment of alveolar-capillary diffusion: The syndrome of "alveolar-capillary block," Am. J. Med., 1951, *11*, 667.

(3) Hamman, L., and Rich, A. R.: Fulminating diffuse interstitial fibrosis of the lungs, Tr. Am. Clin. & Climatol. A., 1935, *51*, 154.

(4) Baldwin, E. deF., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency, Medicine, 1949, *28*, 201.

(5) Stone, D. J., Schwartz, M. J., and Green, R. A.: Fatal pulmonary insufficiency due to radiation effect upon the lung, Am. J. Med., 1956, *21*, 211.

Notes

THE PREDICTED MAXIMAL BREATHING CAPACITY IN OBESE SUBJECTS

The importance and significance of the maximal breathing capacity as a test of pulmonary function have been amply described in the literature. The maximal breathing capacity is usually expressed as a percentage of the predicted maximal breathing capacity. The prediction formula of Baldwin[1] for the 9-liter Collins respirometer or the prediction formula of Motley[2] for the 13.5-liter Collins respirometer is used by many pulmonary function laboratories. Both formulas depend on age and body surface area. The prediction formula of Wright[3] is based on height and age.

In doing studies of the maximal breathing capacity in persons of extreme obesity and subsequent weight loss, it was found that the standard prediction formulas, based on body surface area, for the predicted maximal breathing capacity are of little value in following the change in maximal breathing capacity and other pulmonary function tests that depend on the percentage of predicted maximal breathing capacity or are correlated with it. The maximal breathing capacity is used in comparison with other tests in order to gain additional information. Gaensler[4] relates the per cent of predicted vital capacity and the per cent of predicted maximal breathing capacity by means of a ratio called the air velocity index, defined as: per cent predicted MBC/per cent predicted VC, and points out the type of ventilation defect involved. The per cent of predicted vital capacity is dependent on height and age, while the per cent of maximal breathing capacity depends on the body surface area and age. This has led to bizarre results when the air velocity index is correlated with the one-second timed vital capacity of Gaensler.[5]

It was decided, therefore, to convert the formula for the calculation of the predicted maximal breathing capacity from using the observed weight to using ideal weight in determining the body surface area. This gave ideal body surface area values. Ideal weights for individuals by age and stature are found in Davenport[6] and are reproduced in McLester and Darby.[7]

TABLE 1

VENTILATORY FUNCTION DATA IN SUBJECTS WITH OBSERVED WEIGHTS 20 TO 40 POUNDS ABOVE IDEAL WEIGHTS

Number of Subject	Observed Weight			Ideal Weight			Per Cent Predicted Vital Capacity	One Second Timed Vital Capacity
	Weight	Per Cent Predicted Maximal Breathing Capacity	Air Velocity Index	Weight	Per Cent Predicted Maximal Breathing Capacity	Air Velocity Index		
								per cent
1	192	98	0.84	172	102	0.87	117	76
2	203	94	1.24	169	102	1.34	76	86
3	212	82	0.89	183	87	0.95	92	70
4	202	68	0.92	178	71	0.96	74	70
5	208	38	0.46	173	41	0.49	83	45
6	195	82	1.01	172	86	1.06	81	87
7	195	69	0.73	162	75	0.80	94	65
8	200	88	0.85	180	91	0.88	104	75
9	213	33	0.53	180	36	0.58	62	54
10	200	48	0.56	180	49	0.58	85	59
Mean	202	70	0.80	175	74	0.85	87	69

In table 1 may be seen the calculation of the per cent of predicted maximal breathing capacity and air velocity index on the basis of ideal body surface area in 10 subjects who were 20 to 40 pounds above ideal weight. It shows no great differences in the per cent of predicted maximal breathing capacity or in the calculation of the air velocity index.

In table 2 may be seen the calculation of the per cent of predicted maximal breathing capacity and air velocity index on the basis of ideal body surface area in 10 persons who were more than 50

[1] BALDWIN, E. DEF., *et al.*: Medicine, 1948, *27*, 243.

[2] MOTLEY, H. L.: Indust. Med., 1953, *22*, 6.

[3] WRIGHT, G. W., AND FILLEY, G. F.: Am. J. Med., 1951, *10*, 642.

[4] GAENSLER, E. A.: Am. Rev. Tuberc., 1950, *62*, 17.

[5] GAENSLER, E. A.: Am. Rev. Tuberc., 1951, *64*, 256.

[6] DAVENPORT, C. B.: Body Build and Its Inheritance, Publication 329, Carnegie Institute of Washington, 1923.

[7] MCLESTER, J. S., AND DARBY, W. J.: Nutrition and Diet in Health and Disease, ed. 6, W. B. Saunders Company, Philadelphia, 1952, pp. 602-603.

TABLE 2

VENTILATORY FUNCTION DATA IN SUBJECTS WITH OBSERVED WEIGHTS 50 TO 200 POUNDS ABOVE IDEAL WEIGHTS

Number of Subject	Observed Weight			Ideal Weight			Per Cent Predicted Vital Capacity	One-Second Timed Vital Capacity
	Weight	Per Cent Predicted Maximal Breathing Capacity	Air Velocity Index	Weight	Per Cent Predicted Maximal Breathing Capacity	Air Velocity Index		
								per cent
1	370	77	0.83	162	110	1.18	93	87
2	281	65	0.76	138	87	1.01	86	70
3	281	70	0.92	148	91	1.20	76	83
4	223	82	0.96	143	98	1.15	85	75
5	220	95	0.85	152	111	0.99	112	83
6	220	66	1.08	156	77	1.26	61	83
7	211	63	1.03	150	73	1.20	61	93
8	196	84	1.17	143	96	1.33	72	80
9	197	82	0.84	146	92	0.94	98	88
10	200	85	0.88	150	95	0.98	97	74
Mean	240	77	0.93	149	93	1.12	84	82

pounds overweight as compared with ideal weights. It shows how the air velocity index now more clearly reflects the "restrictive" character of obesity.

The significance of the calculation of the predicted maximal breathing capacity using the ideal weight is clearly shown in serial studies after loss of weight in obese patients. By using the observed weight to calculate the predicted maximal breathing capacity, the per cent predicted maximal breathing capacity as determined on different dates and with weight loss will show large changes of the per cent of predicted maximal breathing capacity even though the actual maximal breathing capacity in liters per minute does not change.

To illustrate this:

Patient *S. C.*, a 49-year-old male. His height was 157 cm. On October 18, 1958, his weight was 281 pounds; his calculated body surface area based on observed weight was found to be 2.21 sq. m., and the predicted maximal breathing capacity was calculated to be 134.6 L. per minute. His maximal breathing capacity was found to be 87.2 L. per minute and, therefore, 65 per cent of his predicted maximal breathing capacity. When calculated on ideal weight, his predicted maximal breathing capacity was 99.9 L. per minute and his maximal breathing capacity was 87 per cent of predicted. On re-examination on December 15, 1958, the patient had lost 48 pounds, and his body surface area was now calculated to be 2.04 sq. m. His predicted maximal breathing capacity, based on this surface area, was calculated to be 124.2 L. per minute. His maximal breathing capacity determined on this date was found to be 88.2 L. per minute, or 71 per cent of predicted (observed weight). When calculated for ideal weight, his predicted maximal breathing capacity was found, as before, to be 99.9 L. per minute and, therefore, his maximal breathing capacity was 88 per cent of predicted.

In summary: In obese persons (overweight by more than 40 pounds), the predicted maximal breathing capacity should be calculated by using the ideal weight in determining the body surface area.

GEORGE C. LEINER
SOL ABRAMOWITZ
Cardio-Pulmonary Laboratory
Veterans Administration Hospital
East Orange, New Jersey

April 24, 1959

COMPARATIVE STUDY OF URINE TESTS FOR THE DETECTION OF ISONIAZID

There is a pressing need for objective criteria to determine whether patients are indeed taking prescribed medications on a self-administered basis.[1] This is particularly true in the chemotherapy of ambulatory tuberculous patients, in whom it is often impossible to determine whether observed clinical deterioration is due to loss of drug effect or to failure to take the medication properly and regularly. The problem becomes even more serious in those societies in which language and cultural barriers, coupled with little confidence in modern medicine, cast grave doubt on whether an individual can be entrusted to take daily antimicrobial therapy without direct supervision. Moreover, in study groups in which various antituberculous drug trials are being conducted, the degree of regularity with which the drugs are being taken is crucial for the determination of the effectiveness of drug regimens. When two powerful antituberculous chemotherapy regimens are being compared, such as isoniazid versus isoniazid-PAS, the differences in results could hinge to a large measure on the degree of regularity of the self-administered chemotherapy.

Although a satisfactory test for PAS in urine has been established,[2] detection tests for isoniazid in urine have been too complex for general field use. The first studies by the present writers with isoniazid detection procedures were based on materials and methods developed by two industrial drug companies, The Bayer Chemical Company and Hoffmann-La Roche, Inc. In July, 1957, these two isoniazid-detection procedures were studied and evaluated at both the Navajo-Cornell Field Health Research Project at Many Farms, Arizona, and at The New York Hospital.

According to directions supplied by the manufacturer, the Bayer procedure consists of adding tablet *A*[3] to 2 to 5 ml. of urine, shaking briefly before adding tablet *B*,[4] and then shaking again. Urine positive for isoniazid rapidly turns orange-red, while urine negative for isoniazid turns gray. The result must be read within five minutes after adding the tablets.

The analyzing reagent[5] supplied by Hoffmann-La Roche is ammonium vanadate, packaged in plastic capsules. According to the manufacturer's instructions, 5 ml. of this reagent (the contents of one capsule) are added to 5 ml. of urine. A positive result is indicated by a brownish-red coloration which fades on standing, while a negative reaction produces a gray coloration.

An investigation of the value of these tracer substances on ambulatory patients[6] was carried out on 5 Navajo volunteers who were members of the staff at the Many Farms Clinic. These subjects were either on isoniazid medication at that time or had been so treated recently and, since they continued on their normal routine of occupation and family life, they were considered to represent the conditions encountered in ambulatory patients. None of these subjects were receiving other forms of medication.

The study at The New York Hospital was carried out on 6 tuberculous patients on the isolation ward. These patients were maintained on their usual medication, and only the dosage of isoniazid was varied. The additional drugs included PAS and streptomycin for 2 of the patients, and streptovaracin for the other 4.

Dosages ranging from 100 to 300 mg. of isoniazid were administered, and the urine was tested at various times by both the Bayer and Hoffmann-La Roche techniques, usually two hours, six hours, nine hours, and occasionally twenty-four hours after the specific dose.

The results of the urine detection tests at Many Farms Clinic following isoniazid administration are shown in table 1.

Three Navajo subjects who received 100 mg. of isoniazid showed positive reactions in urine in five of the six tests at two hours and in two of the six tests at six hours.

Four subjects received 200 mg. of isoniazid, and at two hours all eight tests were positive, but at six hours only three of the eight were positive.

On the 300 mg. dose of isoniazid all ten tests performed at two hours were positive. At six hours following the drug administration, seven of ten tests were positive, and at nine hours four of ten were positive.

The response obtained after six hours on all of the dosages was extremely unpredictable. It may be noted from the data, however, that only one negative reaction and six trace reactions were ob-

[1] Fox, W.: Tubercle, 1958, *39*, 269.

[2] Dixon, W., *et al.*: Lancet, 1957, *2*, 871.

[3] Tablet *A*—sodium 1:2 naphthoquinone-4-sulphonate, 0.005 gm.

[4] Tablet *B*—sodium carbonate, anhydrous 0.1 gm.

[5] Hoffmann-La Roche analyzing reagent:

Ammonium vanadate	0.1 per cent
Acetic acid	4 per cent
N/1 sulfuric acid	8 per cent

[6] In an effort to determine the feasibility of using urine brought to the clinic after being collected on a field visit, urine allowed to stand for six hours was re-tested and found to give the same reaction as that originally obtained on the fresh specimen.

TABLE 1

RESULTS OF URINE DETECTION TESTS FOLLOWING ISONIAZID ADMINISTRATION AT MANY FARMS CLINIC

Patient	2 Hours		6 Hours		9 Hours	
	Bayer	Hoffmann-LaRoche	Bayer	Hoffmann-LaRoche	Bayer	Hoffmann-LaRoche
100 Milligrams of Isoniazid						
1	Negative	Positive (trace)	Negative	Negative		
2	Positive (trace)	Positive	Negative	Positive		
3	Positive	Positive	Negative	Positive (trace)		
200 Milligrams of Isoniazid						
1	Positive (trace)	Positive (trace)	Negative	Negative		
2	Positive	Positive	Negative	Positive (trace)		
3	Positive	Positive	Negative	Positive		
4	Positive (trace)	Positive	Negative	Positive		
300 Milligrams of Isoniazid						
1	Positive	Positive (trace)	Negative	Negative	Negative	Negative
2	Positive	Positive	Negative	Positive (trace)	Negative	Negative
3	Positive	Positive	Positive (trace)	Positive	Negative	Positive
4	Positive	Positive	Positive (trace)	Positive	Negative	Positive
5	Positive	Positive	Positive (trace)	Positive	Positive (trace)	Positive

tained out of the twenty-four tests made on urine collected two hours after each dose.

At Many Farms, the Navajo who are on domiciliary isoniazid treatment usually take their medication shortly after awakening at sunrise. Consequently, urine specimens would have to be obtained in the morning if the results of the analysis were to be considered significant enough to ascertain any degree of dependability for the individual patient. In an effort to evaluate the practicability of obtaining early morning specimens from ambulatory patients when ability to follow directions for the self-administration of isoniazid is questionable, a field visit was made to a family camp, and specimens were obtained from three six-year-old children. These patients were on a dosage of 100 mg. per day and had supposedly taken their drugs three to four hours prior to passing their urine. The samples were taken at 9:45 a.m. and were returned to the clinic and tested by the Hoffmann-La Roche (ammonium vanadate) method. However, only one sample was positive. As the testing procedure is only partially reliable, the problem of determining if the other children had taken their isoniazid could not be settled definitely.

On testing the urine of one patient at The New York Hospital before isoniazid had been administered, the Hoffmann-La Roche test produced a color change that would ordinarily be considered a "trace" reaction. This "false positive" response was noted on the two-, six-, and nine-hour specimens, although on subsequent days negative reactions were obtained on this patient using the same reagent. The factors responsible for this discrepancy are not known.

The results of the urine-detection testing procedures on The New York Hospital patients following isoniazid administration are presented in table 2.

Following the 100-mg. dosage of isoniazid, all twenty-four tests were noted to be positive after two hours; at six hours there were fifteen positive and nine negative tests; and at nine hours there were ten positive and fourteen negative tests.

On the 200-mg. dosage, all twenty-four specimens were positive after two hours; only two of the twenty-four tests were negative at six hours;

TABLE 2

RESULTS OF URINE DETECTION TESTS FOLLOWING ISONIAZID ADMINISTRATION AT NEW YORK HOSPITAL

Patient	2 Hours		6 Hours		9 Hours	
	Bayer	Hoffmann-LaRoche	Bayer	Hoffmann-LaRoche	Bayer	Hoffmann-LaRoche
100 Milligrams of Isoniazid						
1(*a*)*	Positive	Positive	Negative	Positive (trace)	Negative	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive
2(*a*)	Positive	Positive	Negative	Negative	Negative	Positive
(*b*)	Positive	Positive	Negative	Positive	Negative	Positive
3(*a*)	Positive	Positive	Positive (trace)	Positive (trace)	Negative	Negative
(*b*)	Positive	Positive	Positive (trace)	Negative	Negative	Negative
4(*a*)	Positive	Positive	Positive (trace)	Positive	Positive (trace)	Positive
(*b*)	Positive	Positive	Positive (trace)	Negative	Negative	Positive (trace)
5(*a*)	Positive	Positive	Positive	Positive	Positive	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Negative	Negative
6(*a*)	Positive (trace)	Positive (trace)	Positive	Positive	Negative	Negative
(*b*)	Positive	Positive	Positive (trace)	Negative	Negative	Negative
200 Milligrams of Isoniazid						
1(*a*)*	Positive	Positive	Positive (trace)	Positive	Negative	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive
2(a)	Positive	Positive	Positive	Positive	Negative	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Negative	Positive (trace)
3(*a*)	Positive	Positive	Positive	Positive	Positive	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Negative	Positive
4(*a*)	Positive	Positive	Positive	Positive	Positive	Positive
(*b*)	Positive	Positive	Positive	Positive (trace)	Positive	Possive
5(*a*)	Positive	Positive	Positive	Positive	Positive	Positive
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive (trace)
6(*a*)	Positive	Positive	Positive (trace)	Negative	Positive (trace)	Negative
(*b*)	Positive	Positive	Positive	Negative	Positive (trace)	Positive (trace)
300 Milligrams of Isoniazid						
1(*a*)*	Positive	Positive	Positive	Positive	Positive (trace)	Positive
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive
2(*a*)	Positive	Positive	Positive	Positive	Negative	Positive
(*b*)	Positive	Positive	Positive (trace)	Positive	Negative	Negative
3(*a*)	Positive (trace)	Positive	Positive	Positive	Positive (trace)	Positive
(*b*)	Positive	Positive	Positive	Positive	Negative	Positive (trace)
4(*a*)	Positive	Positive	Positive	Positive	Positive	Positive
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive
5(*a*)	Positive	Positive	Positive	Positive	Positive	Positive
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive
6(*a*)	Positive	Positive	Positive	Positive (trace)	Positive	Positive (trace)
(*b*)	Positive	Positive	Positive	Positive	Positive	Positive

* Urine detection studies were performed on two separate days represented on table above as (*a*) and (*b*).

and nineteen positive and five negative tests were found at nine hours.

On the 300-mg. dosage, all twenty-four tests were positive after two hours and six hours. At nine hours there were twenty positive tests and four negative tests.

A number of problems of interpretation arose in this study. The color of a positive response was not distinct in all cases, and strongly positive tests were rarely obtained. The positive test also faded on standing. A positive test often resembled the dark color of concentrated urine frequently observed in the desert area of Many Farms, where dehydration is common. This was found to be true with either testing method. Both at the Many Farms Clinic and The New York Hospital, observations were made on urine obtained following the restriction of fluid intake. This concentrated urine was diluted with three parts of water in an effort to decrease the intensity of color, thereby making the positive test more readable. However, this attempt did not appear to aid in the interpretation of the test reaction since the test color is related to the concentration of isoniazid. Furthermore, since the color of the positive response closely resembles that of concentrated urines, it is difficult to arrive at an accurate reading. With both testing procedures the color change was occasionally so slight that the reaction was very questionably positive. These doubtful results were considered as "trace" reactions, along with the weak, but more definitely positive tests.

Even though this study involved a total of only 11 patients, which made statistical analysis inappropriate, nevertheless, several differences between the two testing procedures were noted. The Hoffmann-La Roche solution was more easily utilized. Doubtful reactions were rarely noted with this procedure, as the color change is rapid, and even the slightest color change was detected without having to shake the ingredients. Furthermore, at The New York Hospital the Hoffmann-La Roche method produced the only positive response on 12 specimens, while the Bayer test gave a positive response on only 6 specimens for which the Hoffmann-La Roche test was negative. The data from The New York Hospital indicate that the Hoffmann-La Roche test produced more positive and trace reactions than the Bayer method.

In evaluating the results, a comparison of the information obtained at the two centers indicated that the detection methods were much more useful at The New York Hospital, where all tests were positive within six hours following the administration of 300 mg. of isoniazid. On the same dosage schedule, only seven of ten tests were positive within six hours at Many Farms. These results were most disappointing inasmuch as the 300-mg. single daily dose of isoniazid rather than either the 100- or 200-mg. dose is the most widely used in field work.

It is of interest that Gangadharam and his associates[7] reported similar findings in comparing the Bayer and Hoffmann-La Roche isonizaid detection tests. In addition to the Bayer and Hoffmann-La Roche tests, they also evaluated a so-called indirect N-M (naphthoguinone-mercuric chloride) test. The results of their study clearly showed that the Bayer and Hoffmann-La Roche detection tests were insufficiently sensitive and reliable for field use. Although their combined N-M test was superior to either the Hoffmann-La Roche or the Bayer tests, it was considered too complicated for use in the usual ward laboratory or in the field. A recent study[8] was reported on the use of riboflavin as an indicator of isoniazid ingestion in patients on self-administered drug. The preliminary results showed that, when riboflavin was given in appropriate dosage, concentrations of riboflavin in urine above normal range were detected for eighteen to twenty-four hours. The same investigators are conducting a clinical field trial to determine the effectiveness of riboflavin as a "tracer" substance in the urine when it is combined with isoniazid. Should this riboflavin tracer technique prove useful, it might also be applied to other self-administered drugs.

* * *

Ideally any method for determining whether or not the patient has ingested a given medication must satisfy a number of requirements. The procedure must be totally safe for the patient, and easily carried out. The end-point must be definite and reliable, producing accurate evidence that the drug was ingested up to twenty-four hours prior to the test. The methods utilized in this study were both safe and simple for the patient, and the laboratory determinations entailed a minimum of time and equipment. However, the end-point was not definitive for either procedure, and the tests were frequently not sufficiently sensitive to indicate the small quantities of isoniazid excreted a number of hours after ingestion. Consequently, from the over-all evaluation of the information obtained, it is evident that these chemical detectors are not satisfactory for determining with complete accuracy whether or not the individual patient has taken his prescribed medication. Al-

[7] GANGADHARAM, P., *et al.*: Tubercle, 1958, *39*, 191.

[8] HOBBY, G. L., AND DEUSCHLE, K. W.: Am. Rev. Resp. Dis. 1959, *80*, 415.

though the results appear to be quite uniform for specimens obtained within a few hours after the administration of higher dosages of isoniazid, false positive and doubtful or negative reactions occur with enough frequency to cast some degree of doubt on the individual test.

In summary: Two procedures for testing urine for the presence of isoniazid were evaluated in ambulatory and hospitalized patients. With one of the tests (based on a chemical method using ammonium vanadate), it was possible to detect isoniazid within six hours in both ambulatory and hospitalized patients. However, this isoniazid detection test was unreliable beyond six hours. The second procedure for testing for isoniazid in urine (based on a naphthoquinone reagent) could not be trusted for these patients, even at the six-hour observation point.

RICHARD W. ROBERTS
Cornell Medical Division, Bellevue Hospital
New York 16, New York
KURT W. DEUSCHLE
Department of Public Health and Preventive Medicine
The New York Hospital-Cornell University Medical Center
New York, New York; and
The Many Farms Clinics, Cornell-Navajo Field Health Research Project
Many Farms, Arizona

June 5, 1959

NASAL SWAB CULTURES IN PATIENTS WITH PULMONARY TUBERCULOSIS

The frequent coexistence of bronchiectasis and paranasal sinusitis has been attributed to the seeding of the sinuses during cough with infected material from the bronchopulmonary structures. As evidence of such a mechanism, iodized oil (Lipiodol®) placed into the bronchial tree has been noted to gain access to the sinuses after coughing.[1] The possibility suggested itself that a similar phenomenon might occur during cough in patients with tuberculosis, forcing droplets of bacilli-laden sputum into the nose and sinuses.

In addition, there was the possibility that a patient with tubercle bacilli in the sputum might inhale the infected droplets he expels through coughing. The mucus-coated nasal vibrissae are known to possess an efficient filtering action on bacteria suspended in the inspired air, and are presumably capable of trapping at least some of the tubercle bacilli which thus may be inhaled. Factors such as the above aroused speculation whether cultures taken from the nasal area might prove to be a reliable means of obtaining tubercle bacilli from patients excreting them through cough.

A review of the available literature in the English language failed to reveal any reports on this subject. However, one article was found in the Japanese and one in the Czechoslovakian literature. In one[2] of these studies, 293 cultures of nasal washings were obtained by Japanese investigators on 147 persons exposed to tuberculous patients in the course of their work. Two cultures were found to be positive for tubercle bacilli. For comparison, 174 cultures of pharyngeal washings obtained on 115 persons similarly exposed yielded one culture positive for tubercle bacilli. No positive cultures were obtained from nose and throat washings in 132 examinations of 82 patients from the nontuberculous clinic population. What seemed of special interest was that 28 of 473 cultures of nasal washings performed on 142 tuberculous patients were found to be positive for tubercle bacilli. The pharyngeal washings were found positive in 90 of 361 examinations performed on 139 patients. In none of the patients was any tuberculous involvement of the nasal passages noted.

TABLE 1

NASAL SWAB CULTURES FOR ACID-FAST BACILLI

Patients Number	Sputum Smear	Sputum Culture	Nasal Swab	Pharyngeal Swab
1	− − − −	− − − −	− − −	0
2	− − + + +	+ + + +	− −	0
3	+ + + + −	+ + +	− − −	0
4	− + − +	+ + + −	−	0
5	+ + + + +	+ + + +	− − −	0
6	− − − − −	+ + +	− − −	0
7	+ + + +	+ + + +	− −	0
8	+ + + +	+ + + +	− −	+
9	+ + + +	+ + +	− −	−
10	+ + + + +	+ + + + +	− − −	0
11	+ + + + +	+ + + + +	− − −	0
12	+ + + + +	+ + + + +	− −	−
13	+ + + +	+ + + +	− −	+
14	− − − −	− − − −	− −	−
15	+ + + +	+ + + +	− −	+

\+ represents a positive report.
− represents a negative report.
0 represents examination not made.
Cultures 10, 11, 12 were done on one patient.

In the Czechoslovakian report,[3] 114 patients with pulmonary tuberculosis were examined for the presence of tubercle bacilli in the nasal cavity and nasopharynx. The smears of the secretions were cultivated on Sula's fluid ascitic medium and on Löwenstein egg medium. *M. tuberculosis* was found in only 2.6 per cent of the nasal cavity cases, but in 22.8 per cent of the nasopharyngeal cases.

Because of these unpromising reports, it was decided to limit the preliminary study to 45 examinations on 13 patients newly admitted to the wards because of proved or a strongly suspected diagnosis of pulmonary tuberculosis. These patients had not yet been placed under tuberculous chemotherapy. The material for examination was obtained in accordance with a modification of a method described by Mankiewicz[4] for obtaining pharyngeal cultures. Swabs were made by tying gelatin sponge (Gelfoam[5]) strips to applicator sticks with suture material. After swabbing the area under investigation, the gelatin sponge was

[1] BEESON, P. B.: in *Harrison's Principles of Internal Medicine*, ed. 2, Blakiston Division of McGraw-Hill Book Co., New York, 1954, p. 93.

[2] YASUMOTO, R.: Tohoku J. Exper. Med., 1945, *48*, 285.

[3] KOTYZA, F., AND CELAK, J.: Casop. lek. cesk., 1950, *89*, 131.

[4] MANKIEWICZ, E.: Canad. M. A. J., 1953, *69*, 160.

[5] Manufactured by The Upjohn Company.

digested with sodium hydroxide solution, and the resultant material was cultured on Petragnani and Löwenstein egg yolk media. Slight moistening of the swabs just prior to use was found to be mechanically advantageous. The swabbed material from each nostril and, in later cases, a pharyngeal swab also usually were obtained during one sitting. Three examinations were made at different times on one patient, a known heavy excretor of tubercle bacilli.

The results are summarized in table 1. It will be noted that, in this group of patients, predominantly with tubercle bacilli in the sputum, it was not possible to obtain any positive findings with the use of nasal swabs. In contrast, the pharyngeal swabs agreed with the sputum reports in about three-fourths of the cases in which they were employed, although these too would seem to have afforded a somewhat disappointing correlation. It is of interest that a swab passed only once through a patient's sputum contained in a sputum cup, as a control, was found to be positive by the method employed.

In summary: The employment of nasal swab cultures in patients with pulmonary tuberculosis does not appear to be a promising method for the isolation of tubercle bacilli, as judged from the results of the limited study presented or from the sparse reports in the available literature.

ROBERT D. TREVATHAN
SAMUEL PHILLIPS
Pulmonary Disease Service
V. A. Medical Teaching Group (Kennedy) Hospital
Memphis 15, Tennessee

June 15, 1959.

FAILURE OF DESOXYRIBONUCLEIC ACID FROM MYCOBACTERIA TO INDUCE BACTERIAL TRANSFORMATION[1]

Bacterial transformation using bacterial desoxyribonucleic acid (DNA) as transformation factor has been achieved with Pneumococcus, Streptococcus, Hemophilus, and other bacterial genera. Immunologic properties, drug resistance, virulence, or enzymatic characteristics were used as markers. The present note reports on experimental attempts to induce transformation in mycobacteria with DNA from various strains. While the work was in progress, our attention was called to a paper by Katunuma published (in Japanese) in 1954, which claims that resistance to streptomycin was successfully transmitted to susceptible strains by DNA from streptomycin-resistant organisms.[2] Since the question is of basic interest and since the results obtained in this laboratory are at variance with those of the Japanese writer, they are being published briefly at this time.

The desoxyribonucleic acid was prepared by the method of Tsumita and Chargaff[3] from three-week-old surface cultures of *M. phlei*, *M. smegmatis*, *M. tuberculosis* 607, BCG (strain Phipps), and *M. tuberculosis* strain H37Rv. All of the preparations had characteristic ultraviolet-absorption spectra (maximum: 256 mμ) (figure 1). They were stored in frozen solution at −20°C. and used shortly after preparation.

The mycobacterial strains mentioned above were made resistant to 1,000 γ of isoniazid or 1,000 γ of streptomycin. Preparations of DNA from these strains were brought in contact with susceptible bacteria of the same strains, both in the resting phase and in the stage of rapid multiplication. The DNA in concentrations of 0.001 to 1.0 mg. per ml. was left in contact with the bacteria for periods of 0.5 to 24 hours. The experiments were performed in Sauton medium as well as in liquid Tween®-albumin medium with and without special aeration. All attempts to transfer drug resistance under these conditions were completely negative. With some of the saprophytes the spontaneous mutation rate was high, but in no instance did DNA significantly increase the number of resistant mutants observed.

Using virulence as a marker, attempts were made to transform avirulent tubercle bacilli of the strain H37Ra into virulent variants by exposing them to DNA from the virulent strain, H37Rv. At first, these experiments appeared to be successful. Plating out of H37Ra, ten-day-old Tween-albumin cultures of which had been in contact with DNA from H37Rv for thirty minutes, yielded a few cord-forming colonies which morphologically resembled virulent strains. However, upon subsequent transfers, this apparent characteristic was lost. Similarly, it was not possible to select virulent forms by massive infection of mice with the DNA-treated H37Ra and culturing the organs of these animals at intervals up to sixty days after infection.

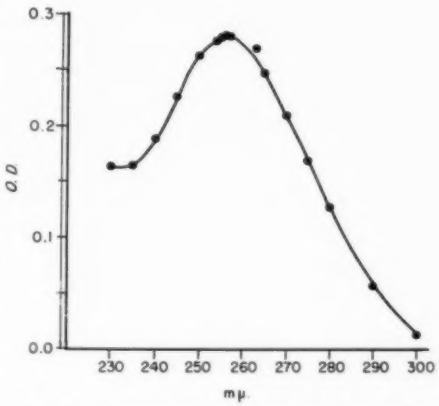

FIG. 1. Absorption spectrum of desoxyribonucleic acid from *M. tuberculosis* strain H37Rv.

Finally, an equally unsuccessful attempt was made to transform nonpigment-forming saprophytes (*M. tuberculosis* 607, *M. smegmatis*) into pigment-forming saprophytes by treating them with DNA from *M. phlei*.

In summary: All attempts failed to induce transformation with desoxyribonucleic acid (DNA) preparations from mycobacteria. Resistance to streptomycin and isoniazid, virulence, and pigment formation were used as genetic markers in these experiments.

HUBERT BLOCH
ALBERT WALTER
Y. YAMAMURA
Department of Microbiology, School of Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania

June 17, 1959

[1] This work was supported by research grant E-2104 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] KATUNUMA, N.: Kekkaku, 1954, *29*, 19.

[3] TSUMITA, T., and CHARGAFF, E.: Biochim. et biophys. acta, 1958, *29*, 568.

SOME OBSERVATIONS ON THE USE OF BENZALKONIUM CHLORIDE (ZEPHIRAN®) IN TUBERCULOSIS BACTERIOLOGY

This laboratory has been studying the effects of benzalkonium chloride (Zephiran®), a quaternary ammonium disinfectant, on various types of mycobacteria. During these studies, certain observations were made which, although not highly relevant to the basic problem of differential susceptibilities, had sufficient practical interest to warrant publication as a separate Note. These observations deal with the applicability of benzalkonium chloride as a decontaminant for specimens to be cultured for *Mycobacterium tuberculosis*.

Hirsch[1] has reported that 0.05 per cent benzalkonium chloride is bactericidal for nonacid-fast bacteria and for saprophytic mycobacteria, but not for tubercle bacilli. On the other hand, levels as low as 0.0002 per cent of benzalkonium chloride in liquid medium exhibited approximately equal bacteriostatic effects on tubercle bacilli as on nonacid-fast bacteria. The present writer has confirmed this with a number of strains of tubercle bacilli and saprophytic mycobacteria (unpublished observations). Patterson[2,3] has proposed a trisodium phosphate benzalkonium chloride digestion and decontamination procedure for sputum. Although this procedure compared favorably in sensitivity with standard digestion procedures, it is not possible to attribute absolutely lethal effects of the benzalkonium chloride treatment, since all clinical specimens had to be treated with some digestant to avoid contamination. Although bactericidal action can be evaluated in cultures grown *in vitro*, the question arises whether bacilli from human pathologic materials are equally resistant to the agent. Possible effects of carry-over of benzalkonium chloride to various culture media also were of interest; Patterson stated that the use of Petragnani medium minimized this problem.

By limiting the present study to resected tuberculous lesions, it was possible to examine the effect of benzalkonium chloride on bacilli, as they occur in man, by comparing treated with untreated aliquots of the same specimens; contamination in carefully handled resected lesions is no problem.

The question of "carry-over" of benzalkonium chloride in concentrations bacteriostatic for *M. tuberculosis* was first considered.

Phospholipids are known to inactivate quaternary ammonium disinfectants, so egg medium should neutralize benzalkonium chloride, whereas a simple agar medium would be less likely to do so. Löwenstein-Jensen egg medium slants and glucose-albumin agar slants (4 ml. per slant) were employed. Four slants of each medium were flooded with 0.1 ml. of each of a series of dilutions of benzalkonium chloride. A pair of tubes of each dilution and medium were permitted to stand for one hour, and the other pair for twenty hours. Each tube was then inoculated with a drop of 1:100 dilution of a twenty-four-hour Dubos Tween®-albumin broth culture of *M. smegmatis*, which is sensitive to the bacteriostatic action of benzalkonium chloride.

One-tenth of a milliliter of 0.25 per cent benzalkonium chloride, after standing twenty hours on the egg slants, had no effect on growth of the *M. smegmatis*. The same type of preparation, after only one hour's standing before inoculation, caused a slight attenuation of growth. One-tenth of a milliliter of 0.05 per cent benzalkonium chloride on egg slants exhibited no residual bacteriostatic effect. On the other hand, even after a twenty-hour equilibration, complete inhibition occurred with 0.1 ml. of 0.05 per cent benzalkonium chloride. No differences were noted between one- and twenty-hour equilibrations.

It was concluded that the "carry-over" effects of benzalkonium chloride from inoculum to egg medium may safely be ignored, but carry-over to agar medium can be significant.

A series of tissue homogenates representing 54 fresh resected lung lesions was examined.

Details of the handling have been described previously.[4] One-tenth milliliter of homogenate was inoculated onto a control tube of Löwenstein-Jensen and one of Petragnani medium. A similar inoculum was mixed with 0.1 ml. of 0.1 per cent benzalkonium chloride, permitted to stand for one hour, and then inoculated onto a Löwenstein-Jensen slant. It was also desired to determine effects on small numbers of bacilli because it is known[5] that the bactericidal action of the quaternary ammonium agents is a function, not only of concentration of the agent, but also of concen-

[1] Hirsch, J. G.: Am. Rev. Tuberc., 1954, *70*, 312.

[2] Patterson, R. A., *et al.*: Am. Rev. Tuberc., 1956, *74*, 284.

[3] Patterson, R. A.: Am. J. Pub. Health, 1956, *46*, 1429.

[4] Wayne, L. G., and Salkin, D.: Am. Rev. Tuberc., 1956, *74*, 376.

[5] Knox, W. E., *et al.*: J. Bact., 1949, *58*, 443.

TABLE 1

GROWTH OF TUBERCLE BACILLI FROM RESECTED LESIONS BEFORE AND AFTER TREATMENT WITH BENZALKONIUM CHLORIDE (ZEPHIRAN)

Specimen	Direct Homogenate			Homogenate Diluted 1:100		
	Löwenstein-Jensen Control	Petragnani Control	Benzalkonium Chloride-Treated	Löwenstein-Jensen Control	Petragnani Control	Benzalkonium Chloride-Treated
668-58	++++	+++	++++	++	++	++
936-58	+++	+++	++++	+	+	+
177-58*	++++	++++	++++	+	+	++
142-59	C	++	++	15	24	20
179-59	++	+++	+++	+	30	40
395-59	+++	++++	+++	0	100	18
999-58	50	9	40	0	0	0
851-58	5	1	6	0	0	5
595-1-58	7	0	0	0	0	0
603-1-58	6	0	0	0	0	0
685-58	0	0	1	0	0	0
38-59	0	0	0	0	3	0
308-59	7	0	12	7	1	1
311-59	1	0	0	0	0	0
483-59†	C	C	30	C	C	0
552-59	24	0	7	0	0	1

Growth is recorded as + to ++++; arabic numerals represent actual colony counts when less than 50 colonies appeared. Contaminated tubes are represented as "C."

* This organism was a "Battey" type of anonymous Mycobacterium.

† All controls carried Staphylococcus, which presumably was present as a secondary invader.

tration of the bacteria. Therefore, control slants were also inoculated with 1:100 dilutions of the homogenates, and similar dilutions were treated with benzalkonium chloride.

Of the 54 lesions examined, 17 yielded cultures positive for tubercle bacilli on one or more tubes. The relative growth from treated and untreated specimens is indicated in table 1. No significant effect of the benzalkonium chloride may be noted on the homogenates or the 1:100 dilutions thereof. It may be concluded that tubercle bacilli grown *in vivo* are no more susceptible than those grown *in vitro* to the bactericidal action of benzalkonium chloride. Some differences in susceptibility of certain "anonymous" mycobacteria are under investigation.

In summary: Benzalkonium chloride at 0.05 per cent final concentration, when used as a decontaminating agent for specimens to be cultured for tubercle bacilli, may be carried over to agar medium in quantities sufficient to exert some bacteriostatic effect. There is no significant "carry-over" effect on egg medium.

Bacilli from resected lesions were just as viable after benzalkonium chloride treatment as were bacilli from untreated control specimens.

LAWRENCE G. WAYNE[6]
Veterans Administration Hospital
San Fernando, California

June 19, 1959

[6] With the technical assistance of Eltha G. Nichols.

HOMOGENIZATION OF VISCOUS SPUTUM

A New Method

The use of a paint shaker for the agitation of sputum and other specimens in examination for tuberculosis has many disadvantages. A paint shaker is expensive, often requires special mounting to reduce noise and vibration, is bulky, and there is a definite hazard from aerosol contamination unless a bacteriologic safety hood is used.

The present writers have adapted a magnetic stirring apparatus for homogenization of specimens which overcomes the objections noted (figure 1).[1]

This small, compact unit consists of a bar magnet securely fastened to the shaft of a rheostat-controlled motor, and both motor and rheostat are completely enclosed in an aluminum housing. To use this instrument, specimens of sputum are collected on three or more consecutive days and transferred into a flat-bottomed, milk dilution bottle with a ground-glass stopper. A digestant consisting of either 3 or 4 per cent aqueous sodium hydroxide, or a solution of one part of Tergitol Penetrant 08[2] and three parts of 4 per cent aqueous sodium hydroxide, as described by LaCava and Ketchum,[3] is added to an equal volume of the specimen, and a sterile Teflon®-covered stirring bar is inserted in the bottle. The bottle is then placed on the magnetic stirrer and the rheostat set for maximal rotation, so that the rapidly revolving Teflon bar creates a deep whirlpool. This is continued for approximately five minutes or until homogenization appears complete. Occasionally, longer periods are required, but never more than twenty-five minutes. The entire specimen is then neutralized, using a sterile solution of 5 per cent aqueous hydrochloric acid with bromeresol purple as an indicator, and is ready for centrifugation. This same method can be used on other specimens, including pus, pleural and gastric fluids, et cetera.

The Teflon rods are sterilized in 5 per cent phenol or they can be autoclaved. They are virtually indestructible and can be reused for long periods. They can be purchased in various sizes, the size used depending on the volume and viscosity of the specimen.

The stirring apparatus requires no oiling and no special care. It is capable of handling a large volume of tuberculous specimens. The use of this machine, along with the digestion mixture (Tergitol and sodium hydroxide), has proved extremely successful in the writers' hands.

FIG. 1. Magnetic stirring apparatus for homogenization of sputum and other specimens.

In summary: A small, compact, inexpensive homogenizer for processing extremely viscous specimens, using a detergent-digestion mixture, has been described.

JOSEPH H. ALLI
THOMAS E. CORCORAN
Laboratory Service
Veterans Administration Center
Des Moines, Iowa

July 27, 1959

[1] "Magne-Stir," manufactured by Labline, Inc., 3070-82 West Grand Avenue, Chicago 22, Illinois.

[2] "Tergitol," manufactured by Carbide & Carbon Chemicals Co., 30 East 42nd Street, New York 17, New York.

[3] LACAVA, F. W., AND KETCHUM, M. E.: Am. Rev. Tubere., 1943, 47, 631.

LONGITUDINAL STUDIES IN EMPHYSEMA

III. Sputum Eosinophilia

Pulmonary emphysema is among the many conditions seen with increasing frequency in a population with an ever-advancing life expectancy. This condition has been classified loosely as a "degenerative" disease, partially at least, because of lack of adequate information on its etiology.

One of the primary confusions about emphysema is the differentiation between the primary or idiopathic form and the secondary type of this process. The former condition appears in mid-life, almost always in males and in those with no allergic background. The secondary condition develops on an extrinsic or intrinsic allergic background. It may appear at any age, in either sex, and the history and/or physical examination reveals the underlying allergic process.

Chronic obstructive pulmonary emphysema ("pseudo-hypertrophic emphysema") is a common cause of dyspnea and asthmatic attacks. It begins with a gradual loss of exercise tolerance and dyspnea on exertion. Patients have more difficulty in the beginning during daylight hours, and nocturnal attacks occur usually only after the disease has progressed. The attack differs materially from that seen in bronchial asthma, in which the dyspnea and wheezing persist for hours, unless specifically treated, and terminate gradually. In contrast, the asthmatic attack due to emphysema is of short duration and is relieved promptly by coughing up plugs of mucus. In a typical case, cough and expectoration occur when the patient retires for the night. Once having cleared his bronchial tubes he goes to sleep, to awaken with cough and dyspnea several hours later. He usually sits on the side of the bed for a few minutes, succeeds in coughing up mucus, is relieved promptly, and is able to sleep again for several hours before further accumulation of mucus initiates another attack. Those who have less advanced disease and who have no nocturnal attacks need a few minutes to an hour or two on arising to clear the respiratory tract and to stop the dyspnea and cough which occur at that time. Emphysema is not an allergic condition. It is associated with atrophy of the alveolar and infundibular walls and subsequent rupture of the walls, and the formation of air-containing cavities of various sizes. There is obstruction of the end arterioles of the bronchial circulation with ultimate development of collateral circulation between the bronchial and pulmonary arterioles. Fibrosis of small bronchioles, especially near the respiratory bronchioles, is a common finding.

The frequency and severity of the nocturnal attacks are determined by the amount of pathologic changes in the larger bronchi, i.e., the severity of the accompanying chronic bronchitis. Examples of emphysema are seen in which there are no cough and no nocturnal attacks, the dyspnea being entirely exertional. The etiology is unknown.

The above-mentioned condition must, of course, be differentiated from pulmonary fibrosis due to tuberculosis, silicosis, asbestosis, et cetera, from Ayerza's disease, so-called acute idiopathic pulmonary fibrosis, tumors obstructing the bronchial tree from within or without, and from aneurysms.

As part of this long-range study of patients with emphysema, it seemed desirable to follow possible changes in the character of the sputum and its microscopic appearance and to relate these changes to the clinical course of the disease. The morning sputum was collected from Monday through Friday of each week for twenty-eight consecutive weeks. Morning sputum rather than twenty-four-hour volumes were used because the subjects were ambulatory, living at home, and wished to avoid the difficulties of more complete collection. In addition, the first sputum raised by an emphysematous patient on arising represents the accumulation in the bronchial tract for several hours and is thus likely to be a representative sample, usually undiluted by saliva. The sputum specimens were examined for color, consistency, homogeneity, presence or absence of mucus or purulent plugs, and were stained with eosin methylene blue. The stained slides were examined for the type and number of leukocytes present, the percentage of eosinophils, and the relative bacterial content.

The 30 patients chosen for this phase of the study all had typical histories of primary or chronic obstructive pulmonary emphysema, classical physical signs, and confirmatory functional studies. All began with exertional dyspnea around the fortieth year without nocturnal dyspnea and with gradual progression of the loss of respiratory reserve. In the more advanced stages, attacks of nocturnal dyspnea occurred which were relieved by coughing up plugs of mucus within a period of five to fifteen minutes. These attacks differed materially from those seen in true bronchial

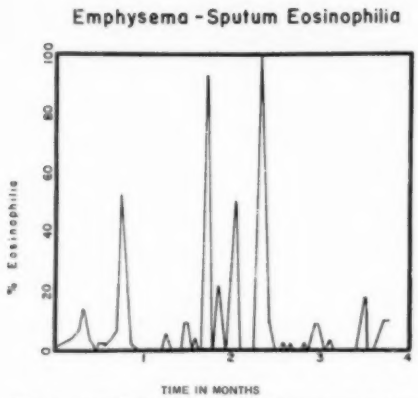

Fig. 1. Graph showing periodic sputum eosinophilia in an emphysematous patient.

asthma. Despite the typical history and appearance of these patients and a negative history of true bronchial asthma, each was tested intradermally with the following inhalant allergens: horse dander, mixed feathers, dust (Endo), timothy pollen, oak pollen, dog dander, alternaria, ragweed pollen, cat dander, and tobacco. None gave significant reactions.

Fifteen of the 30 patients, followed as described above for twenty-eight weeks, showed periodic "showers" of eosinophils in the sputum. These "showers" appeared at intervals from a day or two to three weeks, and lasted usually for a day or two. Between the appearances of these eosinophilic showers, frequently none or only an occasional eosinophil was found. There seemed to be no gross change in the appearance of the sputum during these "showers."

Graphic records of 2 of these patients are shown in figures 1 and 2.

Intensive attempts have been made to correlate these eosinophilic "flares" with an increase in the patient's symptoms or with meteorologic conditions, including temperature, humidity, barometric pressure, wind direction, and mass air movements. Each patient filled out a record form (figure 3) which gave his own impression of his day-to-day progress. It seemed that this method of survey would be less subject to error than daily questioning of the patient, as it would avoid at least one variable, the psychologic attitude of the examiner. Experience with true bronchial asthmatics has demonstrated that marked sputum eosinophilia, such as that seen in these emphysematous patients, is invariably associated with a marked increase in symptoms—in fact, with a severe asthmatic attack. The sputum eosinophilia in these patients could not be correlated either with changes in symptomatology or with any of the meteorologic conditions.

Only 4 of these patients had eosinophilia in the peripheral blood ranging from 4 to 10 per cent. There is no adequate information as yet regarding the fluctuation of blood eosinophilia in relation to that in the sputum. So far as could be determined, none of these patients had any parasitic infestation.

Since it was not possible to implicate inhaled extrinsic allergens as an etiologic factor in the production of the eosinophilia noted, it seemed advisable to investigate the etiologic relationship of common respiratory bacteria. Sputum cultures of enterococcus, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and *E. coli* were grown on synthetic media, and merthiolate-killed vaccines containing 5 billion organisms of each were made. A mixture of these, containing 5 billion organisms per ml., was used for nebulization into the bronchial tree and this was sprayed by intermittent positive pressure (Bennett) for fifteen minutes into one of the writers on two different occasions. No reaction of any kind was observed.

Six of the emphysema patients who had periodic sputum eosinophilia were then studied as follows: A morning sputum sample was obtained and stained for eosinophils; if one or only a few were present, the subject was nebulized as described above for fifteen minutes, using the full-strength mixture. Attention was paid to the occurrence of acute reaction or more delayed increase in symptoms, and sputum was collected each morning for three days. Although 2 of the patients had acute

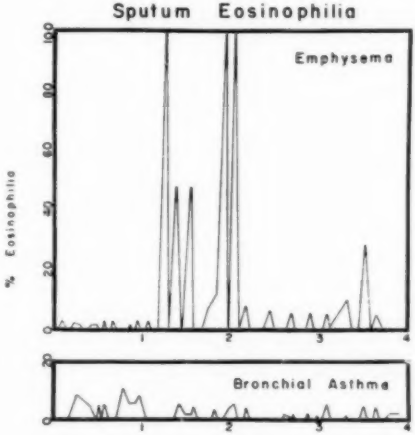

FIG. 2. Graph showing periodic sputum eosinophilia in an emphysematous patient.

DAILY INFORMATION FOR RESEARCH LABORATORY

NAME_____ Dom. Sect._____ Month_____

Normal daily medication_____

Date	Breathing DAY Good	Fair	Poor	NITE Good	Fair	Poor	Additional Medication Yes	Amount	Unusual activity on previous day*
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									

*List any activity of the previous day which was different from your usual routine which might have some connection with how you feel today--such as: Indoors all day - Chilled in the rain - Exposure to dust, fumes, chemicals, etc. - Different medicine - Spent time off the station - Ate meals off station - Smoked more - Drank more - Food did not agree - Reported to Sick Call - Unusual exercise - Excitement or nervous strain - Upset over personal affairs - or anything out of the ordinary.

Fig. 3. Form used by patients to record daily condition.

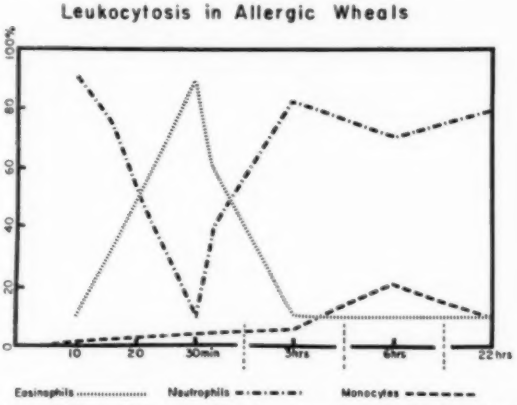

Fig. 4. Differential counts in tissue section of allergic wheals.

bronchial obstruction necessitating hospitalization for forty-eight hours and 2 others had definite, but less acute, exacerbations, no increase in sputum eosinophilia was seen.

* * *

It is generally accepted by allergists that a high concentration of eosinophils in an exudate should be regarded not only as characteristic but as pathognomonic of an allergic reaction and that, if serial examinations fail to demonstrate them in quantity, the process should be regarded as infectious.[1] Local eosinophilia in allergic reactions is of a transient nature, occurring during the first

[1] FEINBERG, S. M.: Allergy in Practice, Year Book Publishers, Inc., Chicago, Illinois, 1946, p. 413.

thirty to forty-five minutes and dropping sharply thereafter. Kline and associates[2] studied the histology of ragweed wheals in tissue removed from human skin at periods of five minutes to twenty-two hours after injection of the antigen. Differential cell counts were made, from which figure 4 was constructed.

It is obvious that tissues examined within forty-five minutes after injection of the antigen would be recognized as allergic; those examined thereafter would be undifferentiated from various degrees of ordinary inflammation. One of the writers (MBC)[3] studied the time of appearance of eosinophils in nasal secretion after introducing a single dose of ragweed pollen into the noses of hay fever patients. They began to appear in ninety minutes, reached a peak around two hours, and gradually disappeared. When a very large dose of dry pollen was introduced or several successive doses were administered during the day, eosinophils were present in the nasal secretion for several days. It was in the light of this observation that the conclusions were drawn from sputum collected for three successive mornings.

As none of these patients had histories indicating the existence of bronchial asthma, attacks typical of the disease, or skin reactions to common allergens, so-called atopic disease can be excluded as well as intrinsic asthma, which has all the clinical and histologic characteristics of an allergic reaction without demonstrable antibodies or antigen. The eosinophilia occurring in these patients then cannot be explained on the basis of allergy unless it be an allergy of a type as yet not understood.

In summary: Fifteen of 30 nonbronchial asthmatic emphysematous patients had "showers" of eosinophils in the sputum at intervals of a few days to a few weeks. These "showers" could not be correlated with patients' symptoms or with meteorologic conditions. The finding of a high eosinophilic content in a secretion is not necessarily evidence of an allergic process which requires specific diagnostic or therapeutic methods for its elucidation.

MILTON B. COLE
JOSEPH V. HAMMEL
ALFRED H. LAWTON
MARYLIN S. MEINDERSMA
SYLVIA B. MACINTYRE
Veterans Administration Center
Bay Pines, Florida

July 1, 1959

[2] KLINE, B. S., *et al.*: J. Allergy, 1932, *3*, 531.
[3] COLE, M. B.: Unpublished report.

Books

ANTIBIOTICS MONOGRAPH NO. 11: MODERN CHEMOTHERAPY OF TUBERCULOSIS. By Roger S. Mitchell and J. Carroll Bell. Pp. 109, Medical Encyclopedia, Inc., New York, 1958. Price, $4.00.

This little book is number 11 in the series, *Antibiotics Monographs*, published under the editorial direction of Henry Welch and Felix Marti-Ibanez. Doctors Mitchell and Bell have combined their talents effectively to produce a clear and concise monograph that is amazingly comprehensive for its size. Although not exhaustive, the bibliography of 510 references is complete enough to serve as an excellent starting point for the student.

The subject matter is well organized into chapters entitled: "History," "Pharmacology," "Modes of Action," "Dosage," "Regimens," "Drug Resistance," "Modern Chemotherapy of Tuberculosis," "Diseases and Conditions Having an Adverse Effect upon the Course of Chemotherapy of Tuberculosis," "Chemotherapy with New and Less Used Drugs," and "Results of Antituberculous Chemotherapy to Date." Most of these topics are discussed in a simple and straightforward manner suitable primarily for the medical student, the general practitioner, and other nonspecialist groups. There are many illustrative charts and tables which summarize very well much of the data discussed in the text.

The sections on pharmacology, modes of action, mechanisms of drug resistance, dosage, and regimens are particularly well conceived. In addition, there is a good discussion of the value of certain ancillary measures, such as bed rest, steroids, collapse therapy, and patient education.

One could take issue with some of the statements made, and point out a few omissions. For instance, tubercle bacilli contain comparatively *small* amounts of endogenous catalase (rather than *large* amounts) when compared with certain other mycobacteria and nocardia. After the description of the reduced virulence of isoniazid-resistant tubercle bacilli, it is stated that a similar phenomenon has been observed with other antituberculous agents and there is cited the attenuation of virulence of organisms grown for prolonged periods in culture media containing Promin.® The mechanism responsible for this attenuation has not been adequately studied but it is undoubtedly quite different from the phenomenon under discussion.

The lack of correlation between isoniazid resistance *in vitro* and the clinical effect of the drug is stressed too much, in the opinion of the reviewer. One almost gains the impression that it makes little difference if the bacilli are susceptible or resistant, although in fact a loss of effectiveness of isoniazid is commonly seen in association with bacillary resistance even though progression of disease may be deterred.

The discussion of drug toxicity is very informative. One should note, however, that, although the ability of cycloserine to increase the tolerated dose of isoniazid in animals is pointed out, the more important excessive central nervous system toxicity of the cycloserine–high-dosage-isoniazid regimen in man is not mentioned.

The section on atypical tuberculosis could be more informative, especially in regard to specific suggestions for therapy.

Finally, the bibliography in a few instances is not completely accurate. References to the simultaneous discovery of isoniazid by three pharmaceutical houses include papers from only two of them. References in support of the concept of a direct relationship between clinical response and degree of inactivation of isoniazid are five in number, but only one of them represents relevant published material. One is a personal communication, one refers to unpublished data of one of the authors, and two cite papers which do not deal with clinical response at all.

These relatively minor faults detract but little from the over-all value of the monograph. For those who desire a brief summary of all of the pertinent facts on the drug treatment of tuberculosis, presented in an easy-to-read, concise manner, it can be highly recommended.

EMANUEL WOLINSKY
Cleveland, Ohio

YEARBOOK OF DRUG THERAPY. Edited by Harry Beckman. Pp. 569, Year Book Publishers Inc., Chicago, 1959. Price, $7.50.

This volume is a collection of abstracts of timely articles from the medical literature dealing with recent developments in drug therapy. The material is organized into twenty-two categories according to general type of disease; therefore, abstracts pertaining to any one new drug may appear under several disease headings. There is, however, no duplication, and articles on the same drug are cross-referenced. A comprehensive subject index and complete author index have been included;

and the printing, binding, paper, and editing are of good quality.

The editor has selected and abstracted articles with the objective of presenting a world-wide cross-section (excluding Iron Curtain countries) of research and opinions on the most important new drugs and new uses for old drugs. A more critical selection would eliminate some trivial studies and make the book smaller and more useful. The editor has interpolated remarks, cross-references, critical comments, and precautionary notes in fine print between the abstracts. Since in many instances the authors of the original articles mention drugs only by trade or generic name, it would be useful if these interpolations were expanded to include comments on the chemical and pharmacologic properties of the drugs. It would also be valuable to indicate whether the agent in question is completely new or, if not, its relationship to known drugs. In a book issued annually, as is this one, opinions of the usefulness of a drug would be expected to swing from one extreme to another; however, the editor by judicious use of comments has contributed a desirable stability.

Each abstract describes briefly the original research and summarizes the results obtained. Opinions and judgments appear to be those of the authors of the original contribution, but occasionally the reader wonders whether they are those of the editor. The heterogeneity of the material presented obviates continuity and detracts seriously from the readability of the book. The condensation and pre-digestion inherent in the abstracting process tend to impair the reader's interest. In spite of these objections, the book should be quite useful to the physician who wishes to make a rapid annual survey of new drugs, but who does not have access to good library facilities. It should also serve him well as a ready reference for dosages, expected results, and side effects. For the medical student or investigator it is a satisfactory entree to the recent literature, and for the specialist it may adequately survey fields other than his primary interest.

EARL H. DEARBORN
Pearl River, New York

PRINCIPLES OF INTERNAL MEDICINE, Third Edition. Edited by T. R. Harrison, Raymond D. Adams, Ivan L. Bennett, William H. Resnik, George W. Thorn, and M. M. Wintrobe. Pp. 1839, The Blakiston Division, McGraw-Hill Book Company, New York, 1958. Price, $18.50.

If this volume contained nothing else of value (which is certainly not the case), the first 500 pages would more than justify its conclusion in every medical library, for in these pages is contained a superb review of the cardinal manifestations of disease and of basic biologic mechanisms important in medicine. This type of compilation of our current understanding of normal and pathologic physiology in relation to symptoms and signs is regrettably lacking in many contemporary textbooks of medicine despite almost universal acknowledgment of the importance of such understanding as a foundation in clinical medicine.

The scope of this section of the book may be indicated by noting that the general topics considered include pain, fever, disturbances in respiration and circulation, indigestion and jaundice, polyuria, oliguria, uremia and edema, alterations in weight, anemia, bleeding and lymphadenopathy, and disorders of nervous function. Considered also are inheritance, development and aging, neoplastic diseases, fluid and electrolyte balance, intermediary metabolism, and electrophysiology.

The remainder of the volume contains descriptions of the various disease states germane to internal medicine. These pieces are, by and large, up to date, authoritative, concise, and scholarly, though it may perhaps be questioned whether infections and infestations, which are accorded 335 pages of text, are presented in realistic proportion to, say, diseases of the cardiovascular system, which occupy 108 pages.

The book concludes with a brief but sorely needed comment on "iatrogenic" disorders which seem to be increasing in frequency in parallel with the potency of our therapy.

All in all, this is a fine textbook of medicine. As indicated above, its consideration of medical fundamentals makes it stand out. It cannot be perused without profit.

J. A. BARONDESS
New York, N. Y.

Obituaries

Frank Ardary Craig

1876–1959

Dr. Frank A. Craig died on May 11, 1959 in his eighty-third year. He had been one of the few surviving founders of the National Tuberculosis Association, and he represented in many ways the fine qualities of those organizers of the first great health agency. Although never a great leader, his steadfast and undaunted will to fight tuberculosis and to work long years for its control or eradication was the stuff which such men as Flick, Biggs, and Trudeau needed to form a durable and successful organization. It is good that Dr. Craig lived to attend the fiftieth anniversary of the founding of the National Association for the Study and Prevention of Tuberculosis at Atlantic City and then to see the virtual control of tuberculosis in the United States and the beginning revival of the old crusading spirit against a myriad of respiratory illnesses.

Dr. Craig was born in Philadelphia on September 28, 1876, and was graduated from the Medical School of the University of Pennsylvania in 1898. He served for most of his life as president of his medical school class, a token of the affection and respect felt for him by his classmates. His first inclination was toward the new specialty of pediatrics because of his love for little children, but after an internship at St. Christopher's Hospital, outpatient work at the Pennsylvania Hospital, and six months study in Vienna, he caught the enthusiasm of Dr. Lawrence Flick's venture in the nearby heavily tuberculous Fifth Ward of the city.

Closing his recently (1902) opened and usually empty office on Thirteenth Street, he joined the staff of the new Henry Phipps Institute in its first month of operation on Pine Street at the same time as did Dr. H. R. M. Landis. He remained to become Associate Director of the Institute and, in his parallel academic career on the medical faculty of the University of Pennsylvania, he progressed steadily from Assistant Instructor in Medicine in 1903 to Emeritus Professor of Clinical Medicine in 1954. As the staff of the Institute was also the visiting staff of the White Haven Sanatorium he served as Visiting Physician to that sanatorium from 1906 and as its Medical Director from 1935 to 1946. He was also on the staff of the Philadelphia General Hospital and the Presbyterian Hospital of Philadelphia for many years.

In addition to being a charter member of the National Tuberculosis Association, he was an active member of a large number of local and national societies, and he served vigorously on the executive committees and the boards of the Pennsylvania Tuberculosis and Health Society and the Philadelphia Tuberculosis and Health Association. He was the first President of the Pennsylvania Trudeau Society. He was a member of the Committee on Housing and Community of President Hoover's Conference on Home Building, a field in which he was well qualified by his studies of the slums of Philadelphia. He was President of the William B. Lake Foundation until his death.

Having served as a member of the Examining Board at Camp Dix during World War I and as physician in charge of the health survey of the police and firemen of the City of Philadelphia in 1920, he became an early advocate of regular annual physical examinations. As a result he edited and helped to write *Diseases of Middle Life*, a work published in two volumes in 1923, which foreshadowed the field of geriatric medicine. He contributed numerous articles to medical journals over a period of fifty years, but his booklets on *Early Days at Phipps*, published at the time of the Fiftieth Anniversary of that Institute, and the *Story of White Haven Sanatorium*, written after that sanatorium closed, were two labors of love. In 1953 he was given a citation from the General Alumni Association of the University of Pennsylvania on Founder's Day. In 1958 he received a medal from the Philadelphia Tuberculosis and Health Association. In May, 1959, he was awarded the Will Ross Medal of the National Tuberculosis Association posthumously. Although he did not survive an operation for carcinoma, he had known of this award for several weeks and had characteristically and modestly written out his appreciation of the honor.

His many friends and co-workers at all levels miss his unique combination of outer gruffness with inner love for all mankind, of the Union League type of political conservatism with ready, outspoken defense of the human rights of the underprivileged, and, above all, of apparent

sternness with unfailing teasing, wit, and good humor. He enlisted in a crusade against tuberculosis and poverty in 1903 and never retired from the battle.

JULIUS L. WILSON

Frederick George Novy
1864–1957

One of the truly great spirits in American medicine, and an honorary member of the American Trudeau Society, Dr. Frederick G. Novy, died on August 8, 1957. He was a pioneer in the new field of bacteriology and one of the last survivors of the group of eager young Americans who went to Europe to work and study in the laboratories of Robert Koch and Louis Pasteur.

Born in Chicago in 1864, the son of Bohemian parents who that year had come to America, he was studious and interested in the world about him. Stimulated to an interest in chemistry and microscopic forms of life by an unusual high school teacher, he earned money by carrying papers and working at night in the public library until funds were available for the purchase of a microscope of his own. Then followed his examination of the swamp lands around Chicago and his first original work, a description of a form of volvox not previously found in this country. This early curiosity, followed by years of solid productivity, was later dramatized by the presentation to him of the 250,000th microscope by Bausch and Lomb.

Specializing in chemistry, Dr. Novy graduated from the University of Michigan in 1886 and soon after accepted an offer to teach in the Department of Organic Chemistry there, later transferring to the Department of Hygiene and Physiological Chemistry headed by Victor C. Vaughn. This was the beginning of a long association and collaboration.

With Vaughn, Novy became interested in the "germ theory," and in 1888 both men went to Europe to take a course given in Koch's laboratory. He brought back with him equipment to teach the new subject, which he did so successfully that it became a requirement in the medical curriculum in the following year, 1890. Among those in the first class was the Professor of Physiology, Henry Sewall, a victim of tuberculosis, who wished to study his own case with the new techniques.

In this country, Dr. Novy acquired prestige as a teacher and investigator. He became Professor of Bacteriology in 1902 and retained this appointment until his retirement in 1935. The quality of his work led to his appointment as a member of the United States commission to study bubonic plague in San Francisco, to an important role in the founding of the Society of American Bacteriologists, and caused him to maintain a close and effective liaison with the Michigan State Department of Health. His earlier work embraced the study of ptomaines (done in collaboration with Dr. Vaughn), investigations in anaerobic organisms, and publication of a chemical textbook for medical students and a *Laboratory Manual of Bacteriology*. In 1907 he isolated a filterable virus from laboratory animals and reported upon it in 1910. In some mysterious way the cultures disappeared, but amazingly enough many years later were discovered and found viable. Although advanced in years, Dr. Novy completed work on this retrieved culture and, with the help of previous carefully made notes, published his findings in 1953.

He is probably best known for his investigations of trypanosomes and spirochetes and, because of his absorbing study of the latter, acquired the nickname of "Spi," a name used affectionately by students and friends for the remainder of his life.

His interest in tuberculosis cropped out at various times: from laboratory procedures, communicability, and prevention in 1894; through studies in respiration in 1925; respiration of the bovine bacillus in 1927; and his Kober Lecture on respiration of microorganisms in 1932. One of the major projects of his long and successful life was his studies on microbic respiration, especially of the tubercle bacillus. Dr. Esmond Long, in his biographic memoir, commented lucidly on this feature of these contributions:

> Their studies gave a clear picture of all of the important features of gas exchange during the growth of tubercle bacilli, and indicated that the slow growth of these organisms in the body could be explained on the basis of respiration and multiplication under conditions of low oxygen tension. Today such studies are carried out with standardized equipment, such as the Warburg apparatus, with which minute amounts of oxygen and carbon dioxide can be measured, but no greater accuracy in uncovering the major aspects of growth is achieved than was attained by Novy and Soule with the more cumbersome but effective devices of Novy and his associates.

In addition to being an original thinker, an astute observer, a meticulous, enthusiastic, and thorough investigator, an ingenious devisor of new apparatus and techniques, he was also a stimulating teacher and a beloved colleague. He was a stern taskmaster and, on occasions in the laboratory, his usually low voice might be heard

in stentorian comment on a student's careless work, infringement of rules, or poor judgment. However, his genuine interest in the student and the twinkle in his eye tempered the sting of his words, and the entire class knew that the comments were for the edification of them all. A linguist of no mean ability who would impishly mispronounce the unsuspecting freshman's name, a master of the half-told story, who enjoyed waiting for the slower portion of the class to catch on, the possessor of an extraordinary memory, he was modest withal. At times as he rocked back and forth on his heels while lecturing, some members of the class would become so absorbed in whether or not he could regain his balance that they completely lost track of what was being said.

As his students and friends would have wished for him, he lived to see and appreciate extraordinary developments in the science he helped create, and, remaining mentally alert, continued to be "a recognized authority and wise counselor, whose advice and assistance were constantly in demand" during the twenty-two years of retirement.

GERTRUDE MITCHELL WILLIS

NOTICES

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

INDEX OF AUTHORS

Abramowitz, Sol, 902
Acharya, B. K., 871
Adhikari, Prasant K., 825
Aldridge, Clifton, 267
Alexander, A. F., Supplement (July), 141
Allen, Albert R., 446
Alli, Joseph H., 914
Altman, David P., 876
American Trudeau Society, 115, 118, 120, 122, 123, 282, 452, 455, 597, 764, 921, 924
Attinger, Ernst O., 38, 46, 53
Auerbach, Oscar, 207

Bates, David V., Supplement (July), 172
Beall, Gildon N., 716
Beck, Frederick, 738
Bell, J. Carroll, 108
Bloch, Hubert, 911
Blount, S. G., Jr., Supplement (July), 128
Bojalil, L. F., 554
Bond, James O., 188
Bowen, John F., 426
Bower, George C., Supplement (July), 207
Bowman, B. U., Jr., 232
Brantigan, Otto C., Supplement (July), 194
Brinkman, Geoffrey L., 732
Briscoe, W. A., Supplement (July), 136
Brissaud, H. E., 326
Brown, W., Supplement (July), 155
Bruhin, H., 559

Cerbón S., J., 554
Chaikof, Leo, 732
Chaves, A. D., 585
Citron, K. M., 167
Clark, Mary E., 744
Clayton, Y. M., 167
Cole, Leon R., 398
Cole, Milton B., 915
Coray, Steven, 264
Corcoran, Thomas E., 914
Corpe, R. F., 388
Crowle, Alfred J., Supplement (July), 153

Darzins, E., 866
Davies, Roberts, 188
Debré, Robert, 326
Des Prez, Roger, 431
Deuschle, Kurt W., 200, 415, 431, 904
Doll, James P., 262
Donohoe, Robert F., 590
Dressler, Sidney H., 111
Duke, C. James, 590
Dunbar, Frank P., 188
Durr, Frederick E., 876

Ebert, Richard V., Supplement (July), 45, 169, 209
Edwards, Lydia B., 747
Eiseman, B., Supplement (July), 147, 155
Ellison, Lois T., 181
Ellison, Robert G., 181

Favez, G., 26
Felton, Frances G., 267
Ferebee, Shirley H., 371
Ferrer, M. Irené, 510
Filley, Giles F., Supplement (July), 213
Fletcher, C. M., 483
Forrest, Elizabeth, 744
Fowler, Ward S., Supplement (July), 118
Frank, N. Robert, 806
Fritts, Harry W., Jr., Supplement (July), 131
Fry, Donald L., Supplement (July), 123

Gaensler, Edward A., Supplement (July), 185
Gale, David, 95
Gallaher, B. Shannon, 181
Garattini, S., 110
Gebauer, Paul W., 6
Gensini, Goffredo, 1
Gerbeaux, J., 326
Green, Robert A., 65, 833, 895
Guld, Johannes, 255

Haapanen, Jaakko H., 1
Hallett, Wilbur Y., 716
Hamilton, W. F., 181
Hamilton, William F., Jr., 181
Hammel, Joseph V., 915
Hardy, Albert V., 188
Harvey, Réjane M., 510
Henderson, Ruth W., 398
Hobby, Gladys L., 274, 415
Hocott, Joe B., Supplement (July), 45
Hollifield, W. C., 587
Horsfall, Frank L., Jr., 315
Horton, Glenn E., 724
Horwitz, Ole, 659
Houston, Charles, Supplement (July), 213
Hoyt, Anson, 216
Hurst, Allan, Supplement (July), 179
Hyatt, Robert E., Supplement (July), 138

Jahn, Richard P., 78
Jameson, A. Gregory, 510
Jordahl, Clarence, 431

Kanai, Koomi, 753
Kapur, Vishwa N., 269
Karpinos, Bernard D., 795

Kass, Irving, 1
Kato, Masahiko, 240, 535
Katz, Sol, 590
Kent, Donald C., 806
Kilburn, Kaye H., 441
King, Edward J., 895
Kirby, William M. M., 716
Knight, Ralph A., 264
Knight, Vernon, 12, 443
Krahl, Vernon E., Supplement (July), 24, 158
Kreis, B., 85
Kress, Milton B., Supplement (July), 194
Kushner, Daniel S., 434
Kusunose, Masamichi, 240
Kwiek, Stanislaw, 257

Lambert, H. P., 648
LaMotte, Irene F., 181
Landis, Francis B., 249
Lasche, Eunice M., 188
Lauener, H., 26
Lawrence, L. Theodore, 575
Lawton, Alfred H., 915
Leiner, George C., 902
Leites, Vera, 89
Lenert, Tulita F., 274
Leonardi, A., 110
Lerner, Ernest N., 188
Levy, David, 587
Lewis, Albert G., Jr., 188
Liebow, Averill A., Supplement (July), 67
Lindgren, Inga, Supplement (July), 185
Lockhart, Elizabeth A., 95
Loosli, Clayton G., Supplement (July), 5
Lowell, Francis C., Supplement (July), 181

McDermott, Walsh, 431
MacIntyre, Sylvia B., 915
McLean, Kenneth H., Supplement (July), 58
McMillen, Shirley, 434
Marcus, Stanley, 264
Marion, Arthur J., 59
Marmion, Thomas, 278
Mason, Carl B., 6
Matsunaga, Kiyoteru, 240, 535
Mauser, Marie, 274
Meindersma, Marylin S., 915
Middlebrook, Gardner, 1, 587
Miki, Katsuji, 240, 535
Miller, Donald B., 825
Mitchell, Roger S., 108, 207; Supplement (July), 2, 213
Moore, Frederick J., 216
Moore, T., 223
Morrissey, John F., 855
Motamedi, Ghassem, 587
Mount, Frank W., 371

Muchmore, Harold G., 267
Mueller, Eugene, Supplement (July), 194
Murray, Francis J., 371
Muschenheim, Carl, 431
Myers, J. Arthur, 100

Nacman, Martin, 111
Nathan, Arthur, 424
Newman, Melvin, M., 806
Nichols, Norman J., 833, 895
Noufflard, H., 326

Oatway, W. H., Jr., 108
Oderr, Charles P., Supplement (July), 104
Orton, S. P., 388

Packalén, Th., 19, 410
Paldino, Rita L., 398
Palmer, Carroll E., 747
Paoletti, R., 110
Parlett, Robert C., 153, 886
Pepys, J., 167
Pérez-Tamayo, Ruy, 554
Perkins, James E., Supplement (October), 138
Petty, T., Supplement (July), 147
Phillips, Samuel, 641, 724, 909
Pierce, John A., Supplement (July), 45
Pizzalato, Philip, Supplement (July), 104
Popper, Hans, 71
Potter, Edith L., Supplement (July), 5
Prez, Roger Des, 431

Raffel, Sidney, 849
Ramsey, Hal H., 267
Rauf, Robert A., 806
Redmond, W. B., 232
Reeves, J. T., Supplement (July), 128
Rehr, Carolyn A., 886
Richert, Joel H., 760
Riddell, R. W., 167
Riemensnider, Dick K., 108
Riley, Edgar A., 426
Robb, C. J., 110
Roberts, Albert, 582
Roberts, Robert W., 904
Robson, J. M., 871
Romansky, Monroe J., 590
Rose, Harold D., 249
Rubin, Ruth C., 855
Runyon, Ernest H., 277
Russell, W. F., Jr., 587

Salkin, David, 59, 447
Schless, James M., 569
Segal, Maurice S., 38, 46, 53
Seinfeld, Edward, 845

Sharman, I. M., 223
Short, E. I., 167
Siebens, Arthur A., 806
Silen, W., Supplement (July), 147, 155
Simpson, David G., 426
Smith, C. Richard, 216
Smith, Donald W., 876
Spivey, C. G., 259
Starr, Paul, 845
Stasko, Irene, 274
Sternlieb, Richard O., 249
Sula, L., 438
Sullivan, F. M., 871

Tabakin, Burton S., 825
Takeya, Kenji, 543
T'ao, J. C., 359
Thompson, J. Robert, 71
Thompson, Millie A., 216
Tirunarayanan, M. O., 559
Tobin, C. E., Supplement (July), 50
Toda, Tadao, 543
Trevathan, Robert D., 909
Tunçman, S., 410
Tysarowski, Wieslaw, 257

United States Public Health Service Tuberculosis Therapy Trials, 371, 627, 757
Uyeda, Charles T., 849

Vaichulis, E. M. K., 262
Vestal, Betty L., 806
Vischer, W. A., 559
Voljavec, B. F., 388

Walter, Albert, 911
Warring, Frederick C., Jr., 445
Wasserman, J., 19, 410
Wayne, Lawrence G., 912
Weiss, David W., 340, 495, 676
West, Ann F., 398
Wharton, Dwight J., 188
White, Arthur C., 12, 443
Wier, James A., 259, 569
Willett, Hilda Pope, 404
Williams, M. Henry, Jr., 689, 700
Wolinsky, Emanuel, 269, 522
Woodruff, C. Eugene, 445
Woolf, C. R., 705
Wunderlich, Gooloo S., 371
Wyatt, John P., Supplement (July), 94

Yamamura, Yuichi, 240, 535, 911
Yamaura, Kenji, 543
Yoshimura, Tetsuya, 543
Youmans, Anne S., 750, 753
Youmans, Guy P., 153, 750, 753

Ziskind, Joseph, Supplement (July), 104
Zohman, Lenore R., 689, 700

INDEX OF SUBJECTS

Aerosol, amphotericin-B used as (Notes), 441
Air
  flow, physics of, in emphysema, Supplement (July), 123
  pollution of, and bronchitis (editorials), 582
Allergy in emphysema, Supplement (July), 181
Alveolar-capillary block in leukemia (case reports), 895
Alveolus
  emphysema of, chronic, in horse, Supplement (July), 141
  proteinosis, pulmonary, of (case reports), 249
Amberson, J. Burns, Lecture, 315
American Trudeau Society
  Chest roentgenogram and chest roentgenographic surveys related to X-ray radiation effects and protection from radiation exposure. Statement of the Committee on Radiation Effects, 115
  Necrology, 122
  Notices, 123, 282, 455, 597, 764, 924
  Obituaries, 120, 452, 453, 921
  "Open-negative" problem. Statement of the Committee on Therapy, 118
Amithiozone resistance and action in mycobacteria, mechanism of, 559
Amphotericin-B as aerosol (Notes), 441
Antituberculous treatment, effectiveness of, tested by direct culture of bacilli in patient's blood (Notes), 85
Armed Forces, Selective Service registrants with tuberculosis, 795
Aspen. *See* United States, Colorado
Aspergillus fumigatus, significance of, in sputum, 167

B663. *See* Phenazine
Bacillus(i)
  acid-fast, atypical (Notes), 434
  tubercle. *See* Tubercle bacillus(i)
Bacteria, transformation of, not induced by desoxyribonucleic acid (Notes), 911
Bacteriophage, temperate, from *Mycobacterium butyricum*, 232
Bellows apparatus in pulmonary function studies, 724
Benzalkonium chloride in tuberculosis bacteriology (Notes), 912
Benzoyl-PAS and PAS, inhibiting isoniazid inactivation in man, 26
Biochemistry in analysis of virulence of tubercle bacilli, 535
Blastomyces dermatitidis and *Histoplasma capsulatum* polysaccharide skin tests on humans (Notes), 264
Blood, direct culture of bacilli in, as drug therapy test (Notes), 85
"Bluing" phenomenon, contamination source in tubercle bacilli cultures (Notes), 95
Books
  Antibiotics Monographs: No. 8. Chloromycetin (Chloramphenicol). By Theodore E. Woodward and Charles L. Wisseman, Jr., 450
  Antibiotics Monographs: No. 9. Penicillin. By Harold L. Hirsh and Lawrence E. Putnam, 450
  Antibiotics Monographs: No. 10. Streptomycin and Dihydrostreptomycin. By Louis Weinstein and N. Joel Ehrenkranz, 450
  Antibiotics Monograph No. 11: Modern Chemotherapy of Tuberculosis. By Roger S. Mitchell and J. Carroll Bell, 919
  Bacterial and Mycotic Infections of Man. Edited by René J. Dubos, 594
  BCG Vaccination Against Tuberculosis. By Sol Roy Rosenthal, 595
  Biología del Cancer. By José Abelló, 595
  Ergebnisse der Tuberkuloseforschung, Vol. XIV. Edited by St. Engel, L. Heilmeyer, J. Hein, E. Uehlinger, 279
  An Experiment in Mental Patient Rehabilitation. By Henry J. Meyer and Edgar F. Borgatta, 450
  John Jacob Abel, M.D. A Collection of Papers by and about Him, 113
  Long-Term Illness. Management of the Chronically Ill Patient. Edited by Michael G. Wohl, 762
  Manual of Chest Clinic Practice in Tropical and Sub-Tropical Countries. By A. J. Benatt, 280
  Principles of Internal Medicine, Third Edition. Edited by T. R. Harrison *et al.*, 920
  Staphylococcal Infections. By Ian Maclean Smith, 113
  Textbook of Medicine (Tenth Edition). Edited by Russell L. Cecil and Robert F. Loeb, 761
  Tumors of the Lungs and Mediastinum. By B. M. Fried, 113
  Veterans Administration Hospitals Number, The Medical Clinics of North America, January, 1959, The Major Pulmonary Diseases. Benjamin B. Wells and Mabe J. Musser, Consulting Editors, 762
  Yearbook of Drug Therapy. Edited by Harry Beckman, 919
  The Year Book of General Surgery—1958-1959 Series. Edited by Michael E. DeBakey, 594

Breathing
energy cost and control of, in chronic pulmonary emphysema, Supplement (July), 131
mechanics of
gas exchange and pulmonary circulation, influence of ventilatory mechanics on, 53
physical properties of lung, 38
respiratory work, 46
Bronchitis
and air pollution (editorial), 582
chronic
as etiologic factor in emphysema, obstructive, Supplement (July), 185
prevalence, nature, and pathogenesis of, 483
syndrome, and emphysema, symposium on, Aspen, Colorado, June 13–15, 1958, Supplement (July), pp. 1–213
Bronchus(i), endo-, hamartoma of (case reports), 65

Cardiopulmonary function
in chronic obstructive emphysema, 689
in pulmonary fibrosis, 700
Case finding in tuberculosis in general population, schools, and hospitals, Supplement (October), 73
Catalase activity of *M. tuberculosis* H37Rv (Notes), 257
Cavity(ies)
"open-negative," the problem of (ATS), 118
tuberculous, gaseous content of, 1
Cell(s)
HeLa, growth characteristics of acid-fast microorganisms other than tubercle bacilli in (Notes), 744
mycobacterial, crude, biologic activity of (Notes), 274
tuberculin-sensitized, inhibition of, *in vitro*, 410
Chemoprophylaxis
in emphysema, pulmonary, obstructive, chronic, 716
of tuberculosis, 648
Supplement (October), 1
Chemotherapy
ambulatory, cycloserine-isoniazid in active tuberculosis (Notes), 89
original, of noncavitary pulmonary tuberculosis, isoniazid and isoniazid-PAS in, 641
of photochromogenic mycobacterial infections, 522
to prevent relapse in patients with noninfectious tuberculosis (correspondence), 108
relapse of tuberculous lesions during and after, Supplement (October), 47
of tuberculosis
hospital and home in, Supplement (October), 23
arrested by reinfection in guinea pigs with, 554
pulmonary, relationship to surgery, Supplement (October), 95
Circulation dynamics in emphysema, pulmonary, during exercise, Supplement (July), 128
Collagen of lung, Supplement (July), 45
Cord factor, toxicity of, mechanism of, 240
Cor pulmonale polycythemia, and idiopathic hypoventilation (case reports), 575
CORRESPONDENCE
Chemotherapy to prevent relapse in patients with noninfectious tuberculosis, 108
Differential uptake of isoniazid-C^{14} by *M. paratuberculosis* susceptible and resistant to isoniazid and hydrogen peroxide, 110
Failure to develop sarcoidosis after the oral ingestion of pine pollen, 760
Further observations on the correlation between isoniazid serum concentrations and therapeutic response in human pulmonary tuberculosis, 108
Historic collection of pneumothorax machines and needles, 278
Pancreas vs. omentum in experimental tuberculosis, 445
Rehabilitation and occupational therapy in tuberculosis hospitals, 445, 446
Rehabilitation of tuberculous patients, 111
Source of scotochromogens, 277
Terminology used for discharges from hospital, 447
Tuberculosis was fashionable in 1759, 110
Culture technique, fibrin-clot, for isolation of tubercle bacilli from pleural exudates (Notes), 438
Cycloserine
-isoniazid
high dosage, in tuberculosis, pulmonary, treatment-failure, chronic (Notes), 269
in tuberculosis therapy, ambulant (Notes), 89

Deborah Sanatorium and Hospital, international symposium of, Philadelphia, Pennsylvania, November 20–22, 1958, Supplement (October), pp. 1–139
Desoxyribonucleic acid
failure to induce bacterial transformation (Notes), 911
as growth stimulant of tubercle bacilli, 866
Diffusing capacity for oxygen during exercise, 806
Discharges from hospital, terminology of (correspondence), 447
Drugs, antituberculous, therapy with paired combinations of, 627

EDITORIALS
Air pollution and bronchitis, 582
Natural history of tuberculosis in the human

body: II. Longitudinal observations imperative, 100
Standardization and stability of purified tuberculin, 255
Elastin of lung, Supplement (July), 45
Emphysema
air-flow physics in, Supplement (July), 123
allergy in, Supplement (July), 181
alveolar, chronic in horse ("heaves"), Supplement (July), 141
Conference
summary of, Supplement (July), 209
and "chronic bronchitis" syndrome, symposium on, Aspen, Colorado, June 13–15, 1958, Supplement (July), pp. 1–213
clinical aspects of, Supplement (July), 169
definition of, Supplement (July), 114
diagnosis of, physical and roentgenographic signs and oximeter test in, 705
diffuse, obstructive, surgery in, 825
experimental, Supplement (July), 147
in guinea pig, Supplement (July), 153
familial, Supplement (July), 179
longitudinal studies in (Notes), 915
lung with variability of behavior within, Supplement (July), 136
macrosection and injection studies of, Supplement (July), 94
in man, natural history of, Supplement (July), 169
microradiography in, Supplement (July), 104
obstructive
bronchitis, chronic, as etiologic factor in, Supplement (July), 185
chronic, cardiopulmonary function in, 689
pathogenesis of, theories of, Supplement (July), 2
pathology of, Supplement (July), 58
pulmonary
chronic
breathing, energy cost and control of, in, Supplement (July), 131
respirators in, 510
circulatory dynamics in, during treadmill exercise, Supplement (July), 128
experimental, Supplement (July), 158
lymphatics in reference to, Supplement (July), 50
and peptic ulcer, Supplement (July), 155
obstructive, chronic, chemoprophylaxis in, 716
surgery in, Supplement (July), 194
and vascular changes, Supplement (July), 67
registry for, Supplement (July), 207
unusual forms of, Supplement (July), 172
ventilation mechanics in, Supplement (July), 118
Eosinophilia, sputum (Notes), 915
Erythromycin in chemoprophylaxis of emphysema, 716
Exudates, pleural, fibrin clot culture technique for isolation of tubercle bacilli from (Notes), 438

Fibrin-clot culture technique for isolation of tubercle bacilli from pleural exudates (Notes), 438
Fibrosis, pulmonary, cardiopulmonary function in, 700
Fitzsimons Army Hospital, tuberculin reaction in tuberculous patients in, 569
Formosa (Taiwan), tuberculosis in, 359

Gas
exchange
and pulmonary circulation, influence of ventilatory mechanics on, 53
and ventilation, respiratory, in chronic pulmonary emphysema, mechanical respirators in, 510
in tuberculous cavities, 1
Gel
diffusion tests for tuberculosis, 886
double-diffusion test for tuberculosis, 153

Hamartoma. *See* Tumors
"Heaves." *See* Emphysema, alveolar, chronic, in horse
HeLa cells. *See* Cell(s)
Hepatitis, pyrazinamide, serum enzymes in, 855
Histoplasma capsulatum and *Blastomyces dermatitidis*, polysaccharide skin tests on humans (Notes), 264
Home
and hospital, in tuberculosis, including chemotherapy of, Supplement (October), 23
Hormones, hydrocortisone acetate ointment in tuberculin skin reaction (Notes), 587
Horse, emphysema, alveolar, chronic, in, Supplement (July), 141
Hospital
discharges, terminology of (correspondence), 447
and home, in tuberculosis, including chemotherapy of, Supplement (October), 23
tuberculosis
case finding in, Supplement (October), 73
vocational rehabilitation, justification of, in, 59
Hydrocortisone. *See* Hormones
Hydrogen peroxide and isoniazid, *M. paratuberculosis* susceptible and resistant to, differential uptake of isoniazid-C^{14} by (correspondence), 110
Hypoventilation, idiopathic, polycythemia, and cor pulmonale (case reports), 575

International Symposium of the Deborah Sanatorium and Hospital, Supplement (October), pp. 1–139
Isoniazid
alone, and with PAS, in original chemotherapy of noncavitary pulmonary tuberculosis, 641
-cycloserine in tuberculosis therapy, ambulant (Notes), 89
-C^{14}, differential uptake of, by *M. paratuberculosis* susceptible and resistant to isoniazid and hydrogen peroxide (correspondence), 110
detection by urine tests (Notes), 904
effect on bacillary metabolism, 404
high-dosage, and cycloserine in tuberculosis, pulmonary, treatment-failure, chronic (Notes), 269
and hydrogen peroxide, *M. paratuberculosis* susceptible and resistant to, differential uptake of isoniazid C^{-14} by (correspondence), 110
ingestion indicated with riboflavin (Notes), 415
inhibition of, in man, by PAS and benzoyl-PAS, 26
paired with other drug combinations, 627
-PAS, effect on thyroid function, 845
-pyrazinamide, hepatotoxicity of, in tuberculosis, 371
-resistant tubercle bacilli in infection of children, 326
serum concentrations and therapeutic response, correlation of, in tuberculosis, pulmonary, in humans (correspondence), 108
-streptovaricin
controlled clinical trial of (Notes), 757
in tuberculosis, pulmonary (Notes), 424, 431
and thiocarbanidin in tuberculosis, pulmonary (Notes), 590
in vitamin E deficiency, 223

Kidney, epithelial cells, sensitivity of, to PPD and other culture filtrate, 410

Lesions
tuberculous
bacteriologic problems of, Supplement (October), 47
healing, pathology of, Supplement (October), 47
relapse of, during and after chemotherapy, duration of drug treatment in, Supplement (October), 47
Leukemia
infiltration causing alveolar-capillary block (case reports), 895
pulmonary involvement in, 833
Leukocyte(s), migration of, inhibition of, specific and nonspecific, 19
Liver
damage by pyrazinamide, serum enzymes in, 855
toxicity of pyrazinamide-isoniazid in tuberculosis, 371
Lung(s)
anatomy, microscopic, of, Supplement (July), 24
circulation of, and gas exchange, influence of ventilatory mechanics on, 53
collagen and elastin of, Supplement (July), 45
diffusing capacity for oxygen during exercise, 806
disease of
chronic, from atypical mycobacterial infections, 188
rheumatoid (case reports), 732
from atypical tubercle bacilli (case reports), 738
emphysema of
chronic, breathing, energy cost and control of, in, Supplement (July), 131
experimental, Supplement (July), 158
and vascular changes, Supplement (July), 67
emphysematous, variability of behavior within, Supplement (July), 136
fibrosis of, cardiopulmonary function in, 700
human
preparation of, for macroscopic and microscopic study, Supplement (July), 114
respiratory portion, pre- and postnatal development of, Supplement (July), 5
insufficiency, chronic, radioactive iodine (I^{131}) in, 181
involvement in leukemia, 833
leukemic infiltration of, causing alveolar-capillary block (case reports), 833
lymphatics of, in reference to emphysema, Supplement (July), 50
physical properties of, 38
proteinosis, alveolar, of (case reports), 249
sarcoidosis of, evolution of (case reports), 71
tuberculosis of, resection in, in Hawaii, 6
Lupus vulgaris cutis, fatality ratio for, 659
Lymphatics, pulmonary, in reference to emphysema, Supplement (July), 50

Maximal breathing capacity in obese subjects (Notes), 902
Maximal expiratory flow rate, apparatus for bedside and office use, 724
Medium(a),
Dubos, penicillin in, instability of (Notes), 262
synthetic, liquid, new, for cultivation of Mycobacterium species (Notes), 267
Meningitis, tuberculous, prognosis and treatment, 388
Metabolism, bacillary, isoniazid effect on, 404

Microorganism(s), acid-fast, other than tubercle bacilli in HeLa cells, growth characteristics of (Notes), 744
Microradiography in emphysema, Supplement (July), 104
Mycobacteriophage
biologic properties of, 543
D-29 inhibition of, with human tubercle bacilli, by serum factor, 12
Mycobacterium(a)
amithiozone resistance and action in, mechanism of, 559
atypical
comparative pathogenicity of, in experimental animals, 876
infections from, pulmonary disease, chronic, from, 188
isolation of, from healthy persons (Notes), 747
cells, crude, biologic activity of (Notes), 274
photochromogenic, infections of, chemotherapy and pathology of, 522
species, new medium, synthetic, liquid, for cultivation of (Notes), 267
Mycobacterium
butyricum, bacteriophage, temperate, from 232
paratuberculosis, susceptible and resistant to isoniazid and hydrogen peroxide, differential uptake of isoniazid-C^{14} by (correspondence), 110
tuberculosis
H37Rv, catalase activity of (Notes), 257
tuberculosis
metabolism of, isoniazid effect on, 404
Mycoses
sarcoidosis
failure to develop, after oral ingestion of pine pollen (correspondence), 760
pulmonary, evolution of (case reports), 71

Navajo, tuberculosis among, 200
Nose, swab cultures in pulmonary tuberculosis (Notes), 909
Notes
Atypical acid-fast bacilli. III. An expanded schema, 434
"Bluing" phenomenon: A source of contamination in cultures of tubercle bacilli, 95
Clinical evaluation of thiocarbanidin and isoniazid in the treatment of pulmonary tuberculosis, 590
Controlled clinical trial of streptovaricin-isoniazid, 757
Cycloserine-isoniazid in the ambulatory treatment of active tuberculosis after failure of previous chemotherapy, 89
Difference in response of four strains of mice to immunization against tuberculous infection, 753
Effect of topical hydrocortisone acetate ointment on tuberculin skin reaction, 587
Effectiveness of antituberculous treatments tested by direct culture of the bacilli in the patient's blood, 85
Evaluation of cycloserine with high dosage of isoniazid in chronic treatment-failure pulmonary tuberculosis, 269
Factor in serum albumin which prevents the inhibition of propagation of D-29 mycobacteriophage by Tween, 443
Fibrin-clot culture technique for isolation of tubercle bacilli from pleural exudates, 438
Further observations on the biologic activity of crude mycobacterial cells, 274
Growth characteristics of acid-fast microorganisms other than tubercle bacilli in HeLa cells, 744
Histoplasma capsulatum and *Blastomyces dermatitidis* polysaccharide skin tests on humans, 264
Innocuousness and possible therapeutic use of aerosol amphotericin-B, 441
Instability of penicillin in Dubos media, 262
Isolation of "atypical" mycobacteria from healthy persons, 747
New liquid synthetic medium for the cultivation of Mycobacterium species, 267
Relationship of sex to the susceptibility of normal and immunized mice to tuberculosis, 750
Simple paper strip urine test for para-aminosalicylic acid, 585
Streptovaricin alone in the treatment of active pulmonary tuberculosis, 426
Streptovaricin and isoniazid in the treatment of pulmonary tuberculosis, 424, 431
Studies of the catalase activity of *Mycobacterium tuberculosis* strain H37Rv, 257
Tuberculous polyserositis, 259
Use of riboflavin as an indicator of isoniazid ingestion in self-medicated patients, 415

Obesity, maximal breathing capacity in (Notes), 902
Obituaries
Brueckner, Harold H., 120
Craig, Frank Ardary, 921
Dahlstrom, Arthur W., 120
McConkey, Mack, 121
Marcy, C. Howard, 452
Novy, Frederick George, 922
Thayer, Lyman L., 120
Wood, Lawrence E., 453
Occupational therapy and rehabilitation in tuberculosis hospitals (Correspondence), 445, 446

Omentum vs. pancreas in tuberculosis, experimental (correspondence), 445
Oximeter test in emphysema, diagnosis of, 705
Oxygen diffusing capacity during exercise, 806

Pancreas vs. omentum in tuberculosis, experimental (correspondence), 445
Para-aminosalicylic acid
and benzoyl-PAS, inhibiting isoniazid inactivation in man, 26
-isoniazid
effect on thyroid function, 845
in original chemotherapy of noncavitary pulmonary tuberculosis, 641
paired with other drug combinations, 627
PAS. *See* Para-aminosalicylic acid
Penicillin
instability of, in Dubos media (Notes), 262
susceptibility and virulence of, in *M. tuberculosis*, 849
Peptic ulcer and emphysema, Supplement (July), 155
Phenazine (B663), antituberculous activity of, 871
Pine pollen, failure to develop sarcoidosis after oral ingestion of (correspondence), 760
Pneumothorax machines and needles, historic collection of (correspondence), 278
Polycythemia, idiopathic hypoventilation, and cor pulmonale (case reports), 575
Polysaccharide skin tests, *Blastomyces dermatitidis* and *Histoplasma capsulatum*, on humans (Notes), 264
Polyserositis, tuberculous (Notes), 259
PPD. *See* Tuberculin
Pressure-flow-volume interrelationships in man, Supplement (July), 138
Proteinosis, alveolar, pulmonary (case reports), 249
Pyrazinamide
hepatotoxicity, 371
-induced liver damage by serum enzyme determinations, 855
paired with other drug combinations, 627

Radiation, effects and protection from, in chest roentgenographic surveys (ATS), 115
Radioactive iodine (I^{131}) in pulmonary insufficiency, chronic, 181
Rehabilitation
and occupational therapy in tuberculosis hospitals (correspondence), 445, 446
of tuberculous patients (correspondence), 111
vocational, justification of, in tuberculosis hospital, 59
Relapse
of tuberculous lesions during and after chemotherapy, Supplement (October), 47
in tuberculosis, noninfectious, chemotherapy to prevent (correspondence), 108
Resection in tuberculosis, pulmonary, in Hawaii, 6
Respirators in emphysema, pulmonary, chronic, 510
Respiratory tract, viral infections of, 315
Respiratory work in mechanics of breathing, 46
Rheumatism-like lung disease (case reports), 732
Riboflavin as indicator of isoniazid ingestion (Notes), 415
Roentgenography
of chest, surveys, and X-ray radiation effects and protection (ATS), 115
in emphysema, diagnosis of, 705
microradiography in emphysema, Supplement (July), 104

Sarcoidosis. *See* Mycoses
Schools, tuberculosis case finding in, Supplement (October), 73
Scotochromogens, source of (correspondence), 277
Serum
albumin, interference with inhibitory action of Tween on D-29 mycobacteriophage (Notes), 443
enzymes in pyrazinamide hepatitis, 855
mycobacteriophage-inhibiting factor in, 12
Sex, relationship of, to susceptibility of normal and immunized mice to tuberculosis (Notes), 750
Silicosis and tuberculosis, avian (case reports), 78
Sputum
Aspergillus fumigatus in, significance of, 167
eosinophilia (Notes), 915
viscous, homogenization of (Notes), 914
Streptomycin
paired with other drug combinations, 627
-resistant tubercle bacilli infection of children, 326
Streptovaricin
alone in tuberculosis, pulmonary (Notes), 426
-isoniazid
controlled clinical trial of (Notes), 757
in tuberculosis, pulmonary (Notes), 424, 431
Surgery
in emphysema
diffuse, obstructive, 825
pulmonary, Supplement (July), 194
in tuberculosis
pulmonary, 207
relationship to chemotherapy, bacteriologic status, and pathology, Supplement (October), 95
Symposium on Emphysema and the "Chronic Bronchitis" Syndrome, Aspen, Colorado,

June 13–15, 1958, Supplement (July), pp. 1–213.

Tests
gel
diffusion in tuberculosis, 886
double-diffusion in tuberculosis, 153
Histoplasma capsulatum and *Blastomyces dermatitidis* polysaccharide skin, on humans (Notes), 264
oximeter, in emphysema, diagnosis of, 705
simple paper strip urine, for PAS (Notes), 585
urine, for detection of isoniazid (Notes), 904
Therapy with paired combinations of antituberculous drugs, 627
Thiocarbanidin and isoniazid in tuberculosis, pulmonary (Notes), 590
Thyroid function in patients treated with isoniazid-PAS, 845
Tubercle bacillus(i)
acid-fast microorganisms other than, in HeLa cells, growth characteristics of (Notes), 744
atypical, pulmonary disease from (case reports), 738
"bluing" phenomenon as contamination source in cultures of (Notes), 95
direct culture of, in patient's blood, as drug therapy test (Notes), 85
growth stimulated by desoxyribonucleic acid, 866
human, mycobacteriophage (D-29) inhibited in, by serum factor, 12
isoniazid-resistant strains infecting children, 326
streptomycin-resistant strains infecting children, 326
virulence of
biochemical analysis of, 535
penicillin effect on growth of, 849
wax, immunogenicity of, for mice, 216
Tuberculin
PPD, skin reactivity to, delayed, 398
purified, standardization and stability of (editorial), 255
reaction in tuberculous patients, 569
sensitivity, passive transfer of, 398
cells, inhibition of, *in vitro*, 410
guinea pigs, inhibition of leukocyte migration from, 19
skin reaction, hydrocortisone acetate ointment, topical, in (Notes), 587
Tuberculosis
active, cycloserine-isoniazid in ambulatory treatment of (Notes), 89
avian, and silicosis (case reports), 78
bacteriology, benzalkonium chloride in (Notes), 912
case finding in general population, schools, and hospitals, Supplement (October), 73
chemoprophylaxis of, 648; Supplement (October), 1
drug-arrested, reinfection in guinea pigs with, 554
experimental, omentum vs. pancreas in (correspondence), 445
fashionable in 1759 (correspondence), 110
gel
diffusion tests in, 886
double-diffusion test for, 153
global eradication of, Supplement (October), 138
hospital
and home in, including chemotherapy of, Supplement (October), 23
vocational rehabilitation, justification of, in, 59
infection with, difference in response of four strains of mice to immunization against (Notes), 753
isoniazid-pyrazinamide in, hepatotoxicity of, 371
Tuberculosis
lesions from, relapse of, during and after chemotherapy, Supplement (October), 47
natural history of, in humans, longitudinal, observations imperative (editorial), 100
among the Navajo, 200
noninfectious, chemotherapy in, to prevent relapse (correspondence), 108
patients with, rehabilitation of (correspondence), 111
future problem of; program for control of, Supplement (October), 117
pulmonary
in humans, isoniazid serum concentrations and therapeutic response in, correlation of (correspondence), 108
isoniazid-streptovaricin in (Notes), 424, 431
nasal swab cultures in (Notes), 909
noncavitary, isoniazid and isoniazid-PAS in original chemotherapy of, 641
resection in, in Hawaii, 6
streptovaricin alone in (Notes), 426
surgery in, 207; Supplement (October), 95
thiocarbanidin and isoniazid in (Notes), 590
chronic, treatment-failure, cycloserine and high-dosage isoniazid in (Notes), 269
among Selective Service registrants, 795
susceptibility of normal and immunized mice to, relationship of sex to (Notes), 750
in Taiwan (Formosa), 359
vaccination against, with nonliving vaccines, 340, 495, 676
Tumors, hamartoma, endobronchial (case reports), 65

Tween®, inhibitory action on D-29 mycobacteriophage inhibited by serum albumin (Notes), 443

United States
Colorado, Aspen
"first" conference, postscript to, Supplement (July), 213
Symposium on Emphysema and the "Chronic Bronchitis" Syndrome, June 13–15, 1958, Supplement (July), pp. 1–213
Hawaii, resection for tuberculosis, pulmonary, in, 6
Urine
tests
for detection of isoniazid (Notes), 904
simple paper strip, for PAS (Notes), 585

Vaccination with nonliving vaccines against tuberculosis, 340, 495, 676
Vaccines, nonliving, in vaccination against tuberculosis, 340, 495, 676
Ventilagram, expiratory, 724
Ventilation
and gas exchange, respiratory, in chronic pulmonary emphysema, mechanical respirators in, 510
mechanics in emphysema, Supplement (July), 118
Vessels in emphysema, pulmonary, Supplement (July), 67
Virus infections of respiratory tract, 315
Vital capacities, total and timed, for bedside and office use, 724
Vitamin E deficiency, isoniazid in, 223

Wax, tubercle bacillus, immunogenicity of, for mice, 216

Zephiran®. *See* Benzalkonium chloride.

INDEX OF ABSTRACTS

LIST OF JOURNALS ABSTRACTED

A.M.A. American Journal of Diseases of Children
A.M.A. Archives of Industrial Health
A.M.A. Archives of Internal Medicine
A.M.A. Archives of Pathology
A.M.A. Archives of Surgery
Acta Chirurgica Hellenica
Acta chirurgica scandinavica
Acta medica iranica
Acta medica scandinavica
Acta orthopaedica scandinavica
Acta paediatrica
Acta pathologica et microbiologica scandinavica
Acta radiologica
Acta tuberculosea belgica
Acta tuberculosea scandinavica
Aggiornamenti sulle Malattie da Infezione
American Journal of Clinical Pathology
American Journal of Hygiene
American Journal of the Medical Sciences
American Journal of Medicine
American Journal of Pathology
American Journal of Physiology
American Journal of Public Health and the Nation's Health
American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine
American Journal of Tropical Diseases
Anales de la Catedra de Patologia y Clinica de la Tuberculosis
Anesthesiology
Annales chirurgiae et gynaecologiae Fenniae
Annales de l'Institut Pasteur
Annales de l'Institut Pasteur de Lille
Annales medicinae internae Fenniae
Annales de Pediatrie
Annali dell'Istituto "Carlo Forlanini"
Annali Medici di Sondalo
Annals of Internal Medicine
Annals of Rheumatic Diseases
Annals of Surgery
Antibiotic Medicine and Clinical Therapy
Antibiotics and Chemotherapy
Applied Microbiology
Archeia Hygienis
Archives of Disease in Childhood
Archives of Pediatrics
Archivio di tisiologia
Archivos do Ibit
Archiwum Immunologii i Terapii Doswiadczalnej
Arquivos Brasileiros de Medicina Naval
Arquivos Mineiros de Leprologia
Arquivos de Tisiologia
Arthritis and Rheumatism
Australasian Annals of Medicine
Australian Journal of Experimental Biology and Medical Science
Australian and New Zealand Journal of Surgery
Beiträge zur Klinik der Tuberkulose und spezifischen Tuberkulose-Forschung
Boletin da Federacao Brasiliera das Sociedades de Tuberculose
Bratislavské lekárske listy
British Heart Journal
British Journal of Cancer
British Journal of Clinical Pathology
British Journal of Diseases of the Chest
British Journal of Experimental Pathology
British Journal of Industrial Medicine
British Journal of Pharmacology and Chemotherapy
British Journal of Preventive & Social Medicine
British Journal of Radiology
British Journal of Surgery
British Journal of Tuberculosis and Diseases of the Chest
British Medical Journal
Bulletin d'Information du Ministere de la Sante Publique et de la Population
Bulletin of the Johns Hopkins Hospital
Bulletin de l'Union internationale contre la tuberculose
Bulletin of the World Health Organization
California Medicine
Canadian Journal of Public Health
Canadian Medical Association Journal
Cancer
Cancer Research
Časopis lékarů českých
Central African Journal of Medicine
Chest Disease Index and Abstracts
Chinese Journal of Internal Medicine
Chinese Journal of Surgery
Chinese Journal of Tuberculosis
Chinese Medical Journal
Circulation
Circulation Research
Clinical Science
Clinics of Respiratory Organs
Danish Medical Bulletin
Deutsche medizinische Wochenschrift
Diseases of the Chest
Duodecim
Fortschritte auf dem Gebiete der Röntgenstrahlen vereinigt mit Röntgenpraxis

Ftiziologia
Giornale italiano della tuberculosi
Giornale di medicina e tisiologia
Gruzlica
Helleniki Iatriki
Hoja Tisiologica
Indian Journal of Medical Research
Indian Journal of Tuberculosis
Indian Medical Journal
Les Instantanes Medicaux
International Journal of Health Education
International Journal of Leprosy
Japanese Journal of Bacteriology
Japanese Journal of Clinical Tuberculosis
Japanese Journal of Experimental Medicine
Japanese Journal of Thoracic Surgery
Japanese Journal of Tuberculosis
Journal of Allergy
Journal of the American Medical Association
Journal of the American Veterinary Medical Association
Journal of Applied Physiology
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Bone and Joint Surgery
Journal of Chronic Diseases
Journal of Clinical Investigation
Journal of Clinical Pathology
Journal of Experimental Medicine
Journal of the Faculty of Radiologists
Journal français de médecine et chirurgie thoraciques
Journal of General Microbiology
Journal of Gerontology
Journal of Hygiene
Journal of Laboratory and Clinical Medicine
Journal of the Louisiana State Medical Society
Journal of the National Cancer Institute
Journal of the National Medical Association
Journal of Pathology and Bacteriology
Journal of Pediatrics
Journal of Pharmacology and Experimental Therapeutics
Journal de Pneumo-Phtisiologie de l'Afrique du Nord
Journal of the Royal Institute of Public Health and Hygiene
Journal of Thoracic Surgery
Kekkaku
Khirurgija
Klinische Wochenschrift
Laboratornoe delo
Lancet
Laval médical
Lékařské Obzor
Lille Medicale
Lotta contro la tubercolosi
Medical Journal of Australia
Medicina interna
Medicina del lavoro
Medicine
Michigan Health
Microbiologia, Parazitologia, Epidemiologia
Mikrobiologija
Minerva medica
Mississippi Valley Medical Journal
National Medical Journal of China
New England Journal of Medicine
New York State Journal of Medicine
New Zealand Medical Journal
Nordisk medicin
Notiziario dell'Istituto Vaccinogino Antitubercolare
Pediatrics
Postepy Higieny i Medycyny Doswiadczalnej
Poumon et le Coeur
Presse médicale
Problemy Tuberkuleza
Proceedings of the Royal Society of Medicine
Proceedings of the Society for Experimental Biology and Medicine
Proceedings of the Staff Meetings of the Mayo Clinic
Public Health Reports
Quarterly Journal of Medicine
Radiology
Recent Advances in Tuberculosis Research
Referatovy Vyber z Tuberkulosy a Pneumologie
Review of Czechoslovak Medicine
Revista Brasileira de Medicina
Revista Brazileira de Tuberculose e Doencas Toracicas
Revista medica da aeuronautica
Revista mexicana de tuberculosis
Revista paulista de medicina e tisiologia
Revista pernambucana de tisiologia e doencas toracicas
Revue belge de pathologie et de médecine expérimentale
Revue de la tuberculose
Rozhledy v tuberkulose
Rumanian Medical Review
Saishin Igaku
Schweizerische medizinische Wochenschrift
Schweizerische Zeitschrift für Tuberkulose
Scottish Medical Journal
Sea View Hospital Bulletin
Semaine des hôpitaux de Paris
Semaine medicale
Shinryo Rinshō
South African Medical Journal
Sportarztliche Praxis

Statistical Bulletin of the Metropolitan Life Insurance Company
Surgery
Surgery, Gynecology and Obstetrics
Thorax
Tórax
Tubercle
Tuberculology
Tuberculoza
Tuberculozis
Tuberkulosearzt
Wiener klinische Wochenschrift
Wiener medizinische Wochenschrift
World Health Organization Newsletter
Yale Journal of Biology and Medicine
Zeitschrift für Tuberkulose
Zentralblatt für die gesamte Tuberkuloseforschung

Abouav, J., 776
Abraham, E., 288
Adelson, L., 768
Adiao, A. C., 134
Adler, D., 466
Affeldt, J. E., 288
Aho, A., 127, 619
Aiboshi, I., 606
Albrecht, K. F., 474
Albuquerque, A. F. R. de, 615
Aliperta, A., 479
Allen, C., 314
Almeida, F. das N., 146
Altmann, V., 472
Alvarez, J., 458
Amdur, M. O., 314
Amos, J. A. S., 136
Andrejew, A., 784
Andrews, A. H., 619
Andrews, B. E., 141
Anzano, O., 190
Aoki, M., 794
Aoki, T., 137
Arcidiacono, G., 133
Arcos Perez, M., 460
Arduino, E., 772
Aromstam, E. M., 475
Arrigo, S. D', 133
Ask-Upmark, E., 614
Asp, K., 477
Assis, A. de., 618
Atkinson, G. R., 778
Autio, L., 622
Avegno, R. P., 303
Avery, M. E., 791
Awataguchi, S., 772
Azzario, P., 306

Baba, C., 768
Baba, R., 782
Bacon, A. P. C., 131
Bahnson, H. T., 295
Baird, J. A., 465
Bang, N., 131
Barach, A. L., 478
Barcia, A., 137
Barrett, N. R., 461
Barrett, R. J., 472
Bartter, F. C., 604
Bassford, P. S., 778
Bastrup-Madsen, P., 287
Bauerjee, D., 463
Beall, A. C., 469
Beck, A., 791
Beck, G. J., 478
Becker, R. F., 313
Becklake, M. R., 310
Bedford, J., 285
Behar, A. J., 793
Behrer, R., 466
Belin, J., 774
Belli, I., 302
Bendowski, B., 287
Benians, R. G., 146
Bentzon, M. W., 301
Berdnikoff, C., 606
Berendes, H., 771
Berg, O. A., 150
Bergenstal, D. M., 128
Bergeron, A., 774
Berlyne, G. M., 310
Bernatz, P. E., 771
Berra, A., 311
Berry, E. H. J., 460
Bertrand, L., 134
Bickel, G., 286
Bickel, H. H., 767
Bickerman, H. A., 478
Biddle, A., 144
Biozzi, G., 794
Birkeland, K., 145
Birzan, N., 606
Bishop, H. A., 474
Björkman, S., 605
Black, H., 295
Blades, B., 129
Blanchet, L., 771
Blattner, R. J., 465
Bleiker, M. A., 301
Blumenfeld, H. L., 603
Blundi, E., 770
Bo, G., 480
Böas, A. V., 473, 615
Bock, K., 611
Bodis, I., 768
Bogen, E., 624
Bonnell, J. A., 778
Borrie, J., 136
Bottrill, D. E., 612
Boulanger, E., 617
Bourgeois-Gavardin, M., 610, 788
Bouton, J., 458, 465
Bower, A. G., 288
Boyd, H. A., 462

Bozzetti, G., 470
Bradley, A., 465
Breathnach, C. S., 144
Brechner, V. L., 777
Brenneman, A. R., 788
Brettner, B., 606
Bridges, R. A., 771
British Medical Research Council, 785
Britt, R. L., 459
Brooke, N. H., 462
Brown, C. E., 151
Brown, D., 775
Brown, E., 609
Brown, G., 466
Brown, H. M., 613
Brown, I. W., Jr., 151
Browning, R. H., 607
Bruce, L. G., 145
Bruel, R., 606
Bruhin, H., 307
Brunner, S., 600
Bücherl, E. S., 478
Bücherl, R., 478
Bugyi, B., 291
Bunner, R., 464
Burlá, A., 479
Butzengeiger, K. H., 616
Byron, F. X., 292

Cable, J. V., 464
Calhoun, H. H., 126
Campbell, A. E. R., 145
Campbell, E. J. M., 623
Canetti, G., 305, 476
Cannada, E., 477
Cannon, B., 471
Cantlin, M. L., 295
Cantrell, F. P., 132
Cantrell, J. R., 126
Capel, L. H., 467
Capocardo, A., 477
Cappa, P. M., 480
Caracciolo, M., 309, 467, 482
Carbonara, A., 480
Carlotta, D., 465
Cărpinişan, C., 130
Carr, D. T., 132
Carrera, G. M., 774
Carvalho Filho, R. S., 608
Casarett, L. J., 148
Casati, G., 302
Cazzaniga, C., 306
Cerruti, M. M., 475
Cesaro, A. N., 133
Chai, S., 771
Challen, P. J. R., 285
Chang, M. H., 308
Chang, Y. K., 288
Chanock, R. M., 618
Chao, C. F., 771
Chao, H. Y., 771
Chapman, C. B., 310
Chapman, P. T., 472
Chapman, W., 772
Chauvet, M., 286
Checcacci, L., 480
Chemotherapy Group of the Tuberculosis Prevention Society of Japan, 622
Cheng, C. L., 284
Cherniack, N. S., 784
Cherniack, R., 790
Cherniack, R. M., 789
Chesterman, J. T., 470
Chiku, N., 792
Childs, A. J., 783
Chiu, T. W., 288
Chivers, C. P., 779
Christoforidis, A. J., 607
Chungcharoen, D., 309
Ciobanu, C., 130
Cleere, R. L., 781
Cochrane, A. L., 298
Cohen, R. C., 626
Collins, N. P., 284
Colton, R., 149
Committee for the Study of the Mechanism of Cure in the Chemotherapy of Tuberculosis under the Auspices of the Ministry of Education (of Japan), 786
Committee on the Study of Tuberculin Reaction under the Auspices of the Ministry of Education (of Japan), 300
Condry, R., 126
Conway, H., 777
Cook, M. K., 618
Cooley, D. A., 294
Corazza, L. J., 146
Corbella, E. G., 302
Corda, M., 476
Correll, N. O., 791
Corssen, G., 314
Cortes, R., 137
Cory, R. A. S., 616
Costa, Á. De Lima, 608
Costa, G., 307
Coutel, Y., 473
Creech, O., Jr., 775
Crittenden, I. H., 623
Crofton, J. W., 620
Cruze, K., 292
Culiner, M. M., 776
Curci, G., 307, 480
Curtis, J. K., 308
Curven, M. P., 287

Cuthbert, J., 145
Cuthbert, R. J., 308
Czanik, P., 783

Daddi, G., 146, 476
Dahl, D. J., 601
D'Alotto, V., 466
Daly, J. J., 769
D'Ambrosio, R., 464
Darke, C. S., 769
D'Arrigo, S., 133
Dascomb, H E., 303
Dautrebande, L., 309
Davant, D. C. Perez, 294
David, H. L., 146
Davis, H. D., 130
Day, J. C., 472
De, T. D., 135
de Albuquerque, A. F. R., 615
de Assis, A., 618
de Boni, J. A., 284
Decamp, P. T., 604
Deck, F. W., 475
Decker, H. G., 608
de Fernández, C. Perdomo, 284
Deinhardt, F., 126
de Kore, E. Fogel, 458, 465
De Lima Costa, Á., 608
Delpy, J., 606
Del Torre, L., 289
DeMuth, G. R., 148
Dent, S., 610, 788
Deodato, M., 477
De Paula, A., 770, 781
De Ritis, G., 309, 467, 482
Derriks, R., 617
De Simoni, G., 313
Dickinson, D. G., 148
Dickson, I., 481
diLiberti, C., 143
Dillon, J. B., 777
DiMarco, N., 306
DiMaria, G., 790
Di Paolo, A., 464
Ditlefsen, E. M. Lindseth, 602
Dobkin, A., 610
Dodge, H. J., 781
Dodge, M., 602
Doll, R., 296, 614
Doll, S. G., 288
Domagk, G., 619
Dowling, H. F., 784
Drets, M., 458
Drew, D. M., 138
Drimmie, A. M. T., 308
Dubois, A. B., 309
Dunbar, J. M., 140
Durant, T. M., 309
Dusza, B. T., 771
Duval, L., 298

Edge, J. R., 612
Edwards, G., 143
Edwards, J. E., 289, 462
Eggers, G., Jr., 314
Ehrenhaft, J. L., 136
Eisenberg, G. M., 622
Eisner, H., 460
Eldridge, F., 129
Elliott, G., 466
Ellis, F. H., 767
Ellis, J. T., 603
Emerson, G. L., 767
Emerson, M. S., 767
Emmons, C. W., 604
Enterline, P. E., 141
Escudero Villar, J. M., 290
Espersen, E., 145
Evans, J. A., 475

Fabian, L., 610
Failor, H. J., 289
Falk, A., 291
Falk, H., 778
Faulds, J. S., 778
Fegiz, G., 776
Felson, B., 143, 474
Ferenczy, Gy., 303
Ferenczy, S., 603
Ferguson, M. H., 789
Ferreira, A. B., 601
Ferreira-Berrutti, F., 460
Fesani, F., 470
Feyrter, F., 793
Fiandra, O., 137
Fienberg, R., 130
Filho, B. G., 608
Filho, R. S. Carvalho, 608
Fink, B. R., 310
Fischer, F., 287
Fjeldborg, N., 287
Fletcher, C. M., 139
Flippin, H. F., 622
Flumini, S., 309
Fodor, T., 303
Fogel de Kore, E., 458, 465
Foley, A. R., 298
Forbes, G., 465
Fornara, P., 604
Fovino, G. F. Nai, 289
Fowler, W. S., 604
Francis, R. S. R., 460, 465
Franco, J., 144
Frank, N. R., 791
Frankel, L., 284
French Society for Tuberculosis, 472

Frisk, A., 605
Froman, S., 624
Fromme, D., 476
Fuchs, G., 302
Fuks, M. A., 792
Fukui, S., 137
Fukushima, K., 605
Fuller, F. W., 777
Fumagalli, L. A., 135

Gaál, J., 607
Gadermann, E., 314
Gaffuri, E., 311
Gaitonde, B. B., 468
Galletti, P. -M., 481
Galzerano, G., 468
Gammarrota, V., 482
Gantz, J. A., 144
Gardner, C. C., Jr., 770
Garfield, J., 622
Gartmann, H., 469
Gazzaniga, G., 290
Gebbie, T., 473
Geissen, W., 616
Georgescu, P., 626
Gephart, T., 459
German, J. L., 604
Gernéz-Rieux, C., 132, 784
Gertler, W., 469
Giambrone, I., 477
Giel, C. P., 615
Gierhake, F. W., 472
Giese, M., 314
Gilani, S., 311
Gilson, J. C., 298
Giraldi, N., 146
Girdany, B. R., 461
Gliedman, M. L., 310
Golberg, B., 459
Goldring, D., 466
Golla, H., 469
Good, C. A., 136, 303
Good, R. A., 771
Gordieyeff, V. A., 151
Gordon, R. B., 467
Gorecki, P., 131
Gosset, J., 476
Goto, H., 307
Goto, T., 786
Govier, W., 291
Gowing, N. F. C., 288
Graham, R. M., 459
Grant, R. N., 310
Grassi, C., 476
Gray, P. G., 614
Gray, S. W., 286
Green, C. R., 470
Green, R. W., 778
Gribetz, I., 791
Grieco, A., 148
Griep, W. A., 301
Griffith, W. R., 151
Grill, W., 474
Grob, M., 611
Groom, D., 772
Gross, P., 150
Grosse-Brockhoff, F., 623
Grosset, J., 305
Grove, W. S., 789
Grumbach, Mme F., 788
Guertzenstein, E., 471, 479, 775
Guidi, V., 307
Guin, J. J., 304
Guld, J., 300, 301
Gupta, S. K., 132
Guth, R., 477
Guy, E., 609
Gyurech-Vágó, E., 450

Haber, E., 785
Häberlin, F., 142
Haddy, F. J., 794
Haenszel, W., 472
Haga, Y., 778
Hagihara, Y., 784
Hajnal, T., 303
Hales, D. R., 144
Hall, B. E., 144
Hall, W. H., 311
Hallamaa, T., 601
Haller, J. A., 126
Halpern, B. N., 794
Hamburger, M., 622
Hamilton, W., 609
Hammond, E. C., 139
Hansen, E., 131
Harada, M., 137
Harboe, A., 297
Harken, D. E., 295
Harris, M. S., 612
Harrison, H. N., 471
Hatch, H. B., 604, 774
Hattori, M., 787
Hausler, L., 291
Hay, D., 786
Hayashi, O., 626
Hayden, W. B., 292
Havt, D. B., 475
Heath, D., 462
Hedwall, E., 469
Heidenreich, C., 469
Heikkilä, H., 612
Heimburg, P., 478
Heiner, D. C., 139

Heinivaara, O., 127
Heller, L., 297
Helm, W. H., 127
Helmholz, F. H., Jr., 604
Helwig, F. C., 602
Hemingway, A., 478
Herbst, M., 611
Hering, A. C., 293
Hers, J. F. Ph., 463
Herting, G., 793
Hertz, R., 128
Herxheimer, H., 127
Heyworth, F., 127
Hickish, D. E., 285
Hickling, S., 473
Higaki, H., 787
Higgins, I. T. T., 298, 623
Hilbish, T. F., 128
Hilding, A. C., 481
Hill, A. B., 614
Hillenius, L., 769
Hiltz, J. E., 285, 614
Hinds, J. R., 459
Hirata, H., 621
Hiwatari, K., 778
Hiyama, T., 778
Hodgson, C. H., 289
Hofbauer, J., 302
Hoffman, M. S., 781
Hoffman, R. F., 292
Hoffman, R. G., 313
Holaday, D. A., 310
Holden, H. M., 285
Holland, W. W., 141
Holman, C. W., 142
Holmes-Siedle, M., 131
Honda, M., 783
Hood, R. M., 469
Hopkins, W. A., 286
Horányi, J., 289
Horii, E., 775
Horne, N. W., 620
Hornykiewicz, D., 793
Hoshino, A., 138
Hosier, D. M., 470
Howanietz, L. F., 600
Howard, F. H., 778
Howard, J. G., 794
Howland, W. J., Jr., 303
Hu, H. K., 288
Hua, C. T., 308
Huang, C. S., 294
Hubble, R. H., 791
Hudemann, H., 772
Huebner, R. S., 618
Hueper, W. C., 149
Hughes, J. P., 458
Hugonenq, H., 621
Huhti, E., 135
Hulse, W. F., 609
Humble, J. G., 619
Huntington, R. W., Jr., 614
Hurley, G. A. P., 773
Hurwitz, J. K., 770
Hutás, I., 607
Hyman, M. H., 612

Ibring, G., 611
Ii, G., 766
Iio, M., 768
Ikeda, S., 769
Imai, H., 787
Inoue, I., 307
Irvine, K. N., 138
Ishibashi, S., 787
Ishihara, K., 137
Ishikawa, S., 137
Israel, H. L., 127
Itkin, S. E., 478
Ito, F., 307
Ito, K., 785
Iversen, K., 131
Iwai, K., 793
Iwainsky, H., 475

Jackson, G. G., 784
Jacobs, S., 310
Jalas, T.-L., 612
Jallut, R., 774
Janbon, M., 134
Jawetz, E., 620
Jen, C. Y., 294
Jensen, K. Østerskov, 285
Jesniko, R., 619
Jessamine, A. G., 137
Jinmu, S., 785
Johnson, C. A., 769
Johnson, R. L., Jr., 788
Johnston, K. C., 791
Jude, J. -P., 621
Julkunen, H., 619
Junger, P. W., 465

Kaida, K., 785
Kaipainen, W. J., 140, 619
Kaji, M., 768
Kalayanam, T. S., 463
Källqvist, I., 615
Kalthofen, A., 141
Kanabuchi, I., 772
Kanai, K., 476
Kaneko, Y., 778
Kannaka, H., 784
Kapikian, A. Z., 618
Kapila, C. C., 463
Kapoor, S. C., 773

Kapur, S. C., 463
Kariks, J., 461
Karlson, K. E., 310
Katsuyama, S., 792
Katz, A. M., 296
Katz, M., 128
Kaufman, B. J., 789
Kaul, S., 463
Kavan, E. M., 777
Kawamori, Y., 781
Kazantzis, G., 778
Keasling, H., 609
Keats, A., 789
Keefer, D., 789
Keller, L. B., 144
Keltz, H., 149
Kenealy, W. L., 462
Kennedy, J. H., 770
Kerr, M. R., 140
Kertay, N., 303
Kienholz, M., 481
Kiesewetter, W. B., 461
Kikuth, W., 617
Kilbourne, E. D., 603
Kimsey, L. S., 313
Kincade, G. F., 468
King, E., 778
King, E. J., 778
Kinoshita, I., 775
Kirkpatrick, J. A., Jr., 302
Kirman, B. H., 290
Kissane, J. M., 466
Kitamoto, O., 787
Kitazawa, S., 782
Kitazawa, T., 787
Klarnet, J., 467
Klein, M., 291
Kleinfeld, M., 615
Kligerman, M. M., 143
Knapp, A., 469
Knoblauch, A., 606
Knott, J., 609
Knudsen, A., 467
Koenig, L., 144
Koike, S., 784
Kolb, R., 617
Koler, J. J., 602
Kølle-Jørgensen, P., 136
Konno, K., 312
Kore, I., 458
Kore, E. Fogel de, 458, 465
Kordan, B., 141
Körlof, B., 297
Korpela, A., 296
Korst, D. R., 463
Kotin, P., 778
Kräubig, H., 291
Krementz, E. T., 775
Kubota, C., 775
Kudo, S., 305
Kuhn, L. A., 794
Kumabe, H., 618, 622
Kuper, S. W. A., 300
Kurokawa, N., 784
Kuschinsky, G., 787
Kuzuya, N., 126

Lacombe, A., 479, 775
Lagarde, C., 606
Laing, M. C., 473
Laitinen, H., 777
Lal Kamra, G., 776
Lambert, E. C., 129
Lane, W. Z., 604
Langston, H. T., 469, 791
Laros, C. D., 790
Lascăr, I. C., 606
Lataix, P., 774
Latis, G., 780
Laufer, A., 793
Laurenson, G. R., 464
Laustela, E., 128, 601
Lawrence, M. S., 136
Laws, J. F., 469
Layland, W. R., 769
Leathart, G. L., 779
Leblois, J., 132
Lederer, K., 474
Leidi, F., 476
Lennette, E. H., 467
Lenoir, P., 473
Lepper, M. H., 784
Lester, W., 149
Levis, F., 480
Lewin, R. J., 481
L'Heureux, M., 298
Li, C. N., 284
Liang, K. H., 284
Libermann, D., 788
Lichstein, H. C., 311
Liebich, H., 608
Liebow, A. A., 788
Life Insurance Companies' Institute for Medical Statistics at the Oslo City Hospitals, 615
Lilienfeld, A. M., 474
Lin, T. T., 284
Lincoln, E. M., 134
Linde, H., 623
Linde, L. M., 623
Lindseth Ditlefsen, E. M., 602
Lionakis, B., 286
Lippert, K. M., 135
Lipsett, M. B., 128
Lissner, J., 128
Littmann, M. L., 475
Liu, T. C., 284

Livingston, H. J., 130
Ljung, O., 782
Lobpreis, E. L., 775
Lofstrom, B., 611
Lohr, H., 474
Lombardero, M., 137
Lommans, S. M., 308
Loogen, F., 623, 790
Lopane, P., 467
Lopez Soto, R., 137
Lops, M., 306
Lorber, J., 782
Loring, W. E., 292, 477
Louria, D. B., 603, 604
Luachesi, M., 776
Lucchesi, M., 305
Luckey, A., 618
Lugosi, L., 625
Lukinovich, N., 306
Lüllmann, H., 787
Lunetta, Q., 479
Lurie, A., 623
Lutz, A., 144
Lyon, R. H., 311

Ma, W. S., 308
Maas, G., 772
McAllen, M. K., 127
Maccabe, A. F., 606
MacClure, E., 770
McDonald, J. C., 141
McDonald, J. R., 136, 767
McGrew, E. A., 469
Mackay, J. B., 465
MacLeod, H. M., 620, 786
McNab, G. R., 789
McNerney, J. M., 150
Madden, T. J., 289
Magnus, K., 300, 301
Magnusson, M., 300, 301
Magovern, G. J., 129
Mähönen, H., 127
Makino, S., 772
Makita, K., 783
Mann, B., 604
Mannes, P., 617
Mantel, N., 472
Mariano, J., 479
Markiul, I., 778
Marks, B., 766
Marks, J., 780
Martin, G., 132
Martins, J. D., 772
Martiny, P. J., 136
Maruyama, Y., 470
Mason, C. B., 769
Massa B., 306
Massola, U., 306
Mastropaolo, A., 290
Masufuji, K., 606
Masurel, N., 463
Masuyama, K., 613
Mathews, C. B., 605
Matsubara, S., 774
Matsuda, M., 787
Matsumoto, S., 617, 781
Matsumoto, T., 625, 785
Matsumura, H., 766
Matsuura, R., 785
Maurer, E. A., 288
Maurice, G. L., 133
May, R. D., 126
Mazzei, E., 466
Meda, A., 460
Mehnert, J. H., 776
Mehrhof, E., 148
Meissner, G., 616
Mello, A. S., 465
Mendez, F. L., Jr., 288
Mendonça, L. N. F., 471, 775
Mendy Noriega, F., 460
Mescolini, G., 300
Mestitz, P., 607
Miège, A., 286
Mifuchi, I., 784
Mikami, J., 787
Mikol, E. X., 619
Miller, R. D., 604
Miller, W. F., 788
Millman, I., 311
Miskovits, G., 607
Misra, S. S., 610
Mitchel, J. H., 310
Mitchell, J., 779
Mithoefer, J. C., 477
Miwa, S., 126
Miyabe, J., 787
Miyazaki, S., 781
Mlczoch, F., 144
Moersch, R. N., 767
Moertel, C. G., 771
Montanini, N., 313
Moore, T. C., 135
Moran, T. J., 130
Morellini, M., 303
Moretti, E., 307
Morgan, J. G., 296
Mori, R., 784
Mori, T., 773
Morioka, T., 774
Morisue, T., 785
Morken, D. A., 148
Mörner, J., 297
Morrow, P. E., 148
Morton, W., 781
Mosolygõ, D., 301

Moss, W. T., 794
Mouton, D., 794
Muftic, M. K., 792
Mulder, J., 463
Munk, J., 474
Münz, J., 142
Murakami, N., 793
Muras, O., 304
Murasawa, K., 472
Murata, S., 787
Murohashi, T., 149
Murphy, J. L., 310
Mustard, W. T., 146
Mydlil, F., 612

Nadel, J., 622
Nafici, A. T., 129
Nagata, S., 624
Nagayama, K., 778
Nagelschmidt, G., 778
Nai Fovino, G. F., 289
Naito, H., 786
Nakaizumi, N., 787
Nakajima, M., 476
Nakajima, N., 784
Nakajima, T., 785
Nakamura, T., 600
Nakazono, I., 621
Nasta, M., 626
Nattero, G., 460
Nauman, J. D., 143
Nauth-Misir, T. N., 131
Nava, B. C., 480
Neal, H. S., 472
Nefflin, L., 126
Neill, C. A., 295
Netter, R., 135
Nevius, O. B., 612
Newcombe, C. P., 287
Newton, K. A., 619
Neyra Ramirez, J., 290
Nezu, H., 778
Ngai, S., 310
Ni, Y. K., 308
Nicaise, R., 617
Nicod, J. L., 608
Niles, N. R., 133
Nisbet, H. I. A., 622
Nishida, S., 613
Nissen-Meyer, S., 145
Nitschke, A., 144
Nixon, P. G. F., 287
Nodine, J. H., 477
Noguchi, S., 766
Nome, O., 602
Nomura, T., 787
Nordenstam, H., 605
Norhagen, A., 605
Noriega, F. Mendy, 460
Norimatsu, K., 785
Nozaki, S., 781
Nuetzel, J. A., 144
Nyiredi, G., 603

Obayashi, Y., 305
O'Brien, E. J., 472
Ochi, N., 307, 787
Ogawa, S., 312
Ogawa, T., 149, 307
Ogonuki, M., 782
O'Grady, F., 150
O'Hagan-Ward, F. A., 482
Ohela, K., 140
Okada, H., 300
Okada, Y., 137
Okanishi, J., 617
Olivieri, A., 480
O'Loughlin, B. J., 474
Omland, T., 133
Ono, M., 787
Oppenheimer, M. J., 309
O'Rourke, J., 138
O'Rourke, P. V., 472
Osada, S., 149
Osborne, R. K., 785
Oseasohn, R., 768
Østerskov Jensen, K., 285
O'Sullivan, W. D., 295
Oswald, N. C., 287
Ouvarova, O. A., 482
Ovedoff, D. L., 468

Painter, T. S., 463
Pallotta, G., 309, 313, 467
Palmer, H. D., 132
Paltia, V., 611
Palva, T., 135, 138
Pan, C. T., 296
Pana, C., 305
Papadopoulos, C., 789
Papperman, S. M., 310
Parkulainen, K. V., 611
Parr, E. A., 614
Parrella, M., 297
Parrott, R. H., 618
Pasargiklian, M., 146
Pasha, M. A., 604
Pastor, B. H., 146, 770
Pätiälä, J., 477
Patrecca, L., 464
Paula, A. De, 770, 781
Păunescu, E., 626
Pav, D., 146
Pavilanis, V., 298
Peach, A. M., 600
Pegg, D. E., 619

Peniche, A., 776
Peräsalo, O., 128, 601, 612
Perdomo, C., de Fernández, 284
Peree, Y. R., 129
Perelli, A., 289
Perez, M. Arcos, 460
Perez Davant, D. C., 294
Pernambuco, P., 770
Pernis, B., 480
Perruchio, P., 606
Peters, R. M., 292, 477
Petersen, A. G., 602
Petrányi, G., 768
Petrle, M., 612
Pettet, J. R., 470
Peyman, M. A., 458
Pezza, M., 479
Pfisterer, W. H., 619
Pichot-Jantin, Mme, 473
Pickroth, G., 134
Pigliafredi, F., 306
Pilod, M., 301
Pimpinelli, E., 288
Pines, A., 773
Pinto, C. J., 146
Plotkin, R. F., 476
Polasky, N., 622
Pongor, F., 288
Pontiggia, P., 308
Popa, Gr., 780
Popa, M., 780
Popper, H., 304
Popper, L., 128
Porter, H. R., 608
Pothman, F. -J., 617
Power, M. H., 132
Pracht, W., 787
Prentice, A. I. D., 626
Price, E. B., 128
Priest, R., 617

Quinlan, J. J., 285, 614

Radice, J., 294
Rakower, J., 462
Rakowski, D., 789
Ramirez, J. Neyra, 290
Ramon, P., 132
Ramos, H. P., 473
Rankin, J. D., 791
Rao, G. S., 468
Rapp, G. W., 771
Rasmussen, H. K., 308
Ravin, H. A., 147
Ravitch, M. M., 126
Read, L., 476
Reed, E. S., 140
Reemtsma, K., 775
Reeves, J. T., 622
Reich, S. B., 776
Reichelderfer, T., 618
Reid, D. H. S., 130
Reid, J. D., 464, 473
Rescigno, B., 297, 479, 480
Richards, W. F., 467
Rieben, P. A., 481
Riley, H. D., 459
Rist, N., 788
Ritchie, J. A., 145
Rivarola, A. J., 135
Rivarola, J. E., 135
Robson, A. O., 467
Robson, J. M., 791
Roccaforte, D. S., 776
Rogers, D. E., 603
Rominger, C. J., 295
Ronald, A. C., 145
Roos, B. -E., 127
Ross, J. D., 620
Rossa, G., 308
Rothberg, H., 286
Rotthoff, G., 138
Rowe, A., Jr., 766
Rowe, A. H., 766
Rozner, L., 766
Rubin, E., 466
Rubin, M., 466
Rudstrom, P., 606
Ruppe, J. P., 619
Rusnock, J. R., 461
Ryan, R. F., 775
Rydell, J. R., 777

Saenz, A., Jr., 151
Sahara, S., 784
Saito, M., 126
Saito, S., 783
Saitta, G., 133
Sakurai, H., 307
Salisbury, P. F., 481
Sallinen, P., 616
Salsbury, C. G., 778
Salyer, J. M., 471
Sandor, F., 291
Sandrock, R. S., 467
Sands, J. H., 475
Sapirstein, W., 146
Sarot, I. A., 475
Sartorelli, E., 148
Sasaki, R., 769
Sasaki, S., 311, 791
Sattler, A., 766
Sawaoa, Y., 781
Sawai, T., 149, 307
Sawyer, P. N., 661
Saxén, E., 296, 602

Scalfi, G. F., 308
Schaal, J. A., 288
Schaffner, V. D., 285
Schaub, W., 790
Scheinin, T. M., 292
Scherr, M. S., 284
Scherrer, M., 459
Schmidt, F., 766
Schmidt, H., 131
Schmiedel, A., 306, 621
Schneider, L., 601
Schneirerson, S. J., 128
Scholtze, H., 474
Scholz, D. A., 620
Schreck, K. M., 615
Schrenk, H. H., 140
Schulz, D. M., 475
Schwab, M., 478
Scott, R. F., 466
Seabury, J. H., 303
Sealy, W. C., 151
Sefton, A. J., 615
Seiler, H. E., 298
Selinke, H., 460
Selkon, J. B., 300, 311
Senda, Y., 137
Senis, F., 479
Sensenig, D. M., 136
Seri, I., 783
Shah, R. L., 470
Shao, K., 308
Shapiro, S. W., 608
Sharma, U. C., 610
Sharnoff, J. G., 612
Sharp, J. T., 789
Shedrow, A., 606
Sheehy, T. W., 286
Sheldon, W. H., 771
Sherman, F. E., 460
Sherman, R. S., 475
Sherwood, C. E., 767
Shigematsu, N., 785
Shih, M. H., 294
Shimada, H., 149, 307
Shimano, K., 786
Shimkin, M., 472
Shimomura, Y., 307
Shinde, A. G., 468
Shinohara, H., 766
Shipps, F. C., 302
Shooter, R. A., 287
Sicklick, E., 466
Siebens, A. A., 310
Siegel, D., 475
Sieler, H., 469
Simmons, D. H., 478
Simoni, G. De, 313
Simpson, E., 473
Sindo, C., 778
Singh, P., 776
Sirak, H. D., 470
Skandalakis, J. E., 286
Skinner, M. E. G., 619
Slominski, D., 469
Smith, G., 458
Smith, J. T., 791
Smith, L., 135
Smith, N., 476
Smith, R., 292
Smith, R. B. W., 286
Smith, W. E., 130
Smith, W. W., 151
Sneider, P., 468
Snowden, B. A., 769
Society of Thoracic Surgery (France), 472
Soltys, M. A., 141
Someya, S., 626
Somner, A. R., 620
Sones, M., 127
Soroff, S., 295
Sorrentini, R., 468
Soto, R. Lopez, 137
Souza, R. B. P., 465
Sowa, K., 787
Spencer, F. C., 295
Spina, G., 289, 300, 313
Sproule, B. J., 310
Sprunt, W. H., 292, 477
Staffel, C., 794
Stalder, G., 768
Stanham, J., 137
Stauffer, H. M., 309
Ştefănescu-Mindru, C., 130
Stein, M., 147
Stein, S. C., 477
Steinbach, M., 780
Steinberg, I., 142
Steinitz, K. H., 133
Stenström, R., 602
Stephen, C., 610, 788
Stewart, S. M., 620, 786
Stieve, F. E., 302
Stitt, J. F., 145
Stocks, P., 613
Storniello, G., 305, 313
Stovin, P. G. I., 458
Sulamaa, M., 611
Sullivan, H. E., 126
Suter, E., 313
Swanson, H. A., 143
Sweany, S. K., 794
Sweetman, W. R., 468
Szendi, B., 609
Szöts, I., 289
Szücs, S., 607

Tabari, K., 313
Taido, S., 137
Tajima, Y., 768
Taka, Z., 613
Takahashi, A., 600
Takahashi, U., 137
Takeya, K., 784
Tal, C., 793
Tala, P., 128
Talal, N., 143
Tammeling, G. J., 790
Tanaka, K., 785
Tansini, G., 288
Tapley, N. duV., 143
Taquet, A., 784
Tashiro, K., 126
Taylor, W. J., 295
Thomas, W. A., 466
Thompson, C. A., 132
Thompson, H., 287
Thorburn, W. B., 141
Thrower, W. B., 295
Timmes, J. J., 310
Tirunayaranan, M. O., 307
Toda, T., 784
Todosijczuk, D., 773
Tokunaga, T., 784
Tomalino, D., 285
Tóth, F., 607
Totten, R. S., 130
Toyohara, K., 305, 775
Trappman, R., 140
Treppinger, K., 621
Tsukamura, M., 303, 787
Tucker, W. B., 142
Tuhy, J. E., 133
Tummillio, M., 477
Turner, J. K., 794
Turner, P. P., 620
Turunen, M., 777
Tuttle, W. M., 472

Uchiyama, H., 774
Ueda, S., 606
Ugon, V. A., 285
Ullman, G. E., 313
Urabe, M., 793
Urquhart, K. R., 308
Urushigaki, I., 792
Urushizaki, K., 312
Utahashi, S., 778
Utz, J. P., 604

Vallade, L., 621
van der See, J., 141
Vandiviere, H. M., 151
Vandiviere, M. R., 151
van Vliet, B., 141
Varga, G., 607
Vargosko, A., 618
Vasconcelos, C., 471, 479
Velican, C., 780
Verel, D., 769
Vestal, B. L., 310
Vidal, J., 304
Vieten, H., 138
Vignati, P., 306
Villar, J. M. Escudero, 290
Virgilio, R., 290, 480
Virkkula, L., 612
Vischer, W. A., 307
Vlad, P., 129
Voisin, C., 132
Volk, T. E., 613
Vossschulte, K., 472
Vosti, K. L., 784
Voutilainen, A., 602

Waaler, H., 300, 301
Wachi, T., 625, 785
Wada, H., 785
Wada, T., 606, 768
Waddell, W. R., 471
Wallgren, G. R., 611
Wang, T. H., 294
Ward, R., 476
Warner, C. L., 459
Watanabe, T., 613
Watkins, D. H., 137
Watkins, E., Jr., 293
Wayl, P., 462
Weber, M., 474
Webster, E. W., 783
Weinstein, H. J., 602
Weiss, V. W., 622
Weiss, W., 477
Welin, S., 610
Wells, P. O., 140
Welstead, A. G., 298
Wenzl, M., 766
Westlake, E. K., 623
Westrick, M. L., 150
Wheeler, J. E., 476
White, H., 286
Whitfield, A. G. W., 462
Wholey, M. H., 136
Wicker, E. H., 140
Wilk, S. P., 475
Wilkins, A. J. W., 782
Will, D., 624
Willett, H. P., 311
Williams, D. A., 127
Williams, R. G., 134, 299
Williams, W. J., 297
Williamson, J., 298
Willis, H. S., 151

Wilson, C. W., 619
Wilson, H. N., 613
Wilson, J. L., 148
Wilson, J. S., 141
Wilson, L. L., 464
Wilson, M., 309
Wilson, W. R., 769
Winblad, J. N., 775
Winbury, M., 291
Winters, W., 309
Wise, R. A., 468
Witz, Mme M. A., 144
Wolf, J., 291
Wooiner, L. B., 771
Wright, R. R., 465
Wu, H. N., 605, 788
Wyllie, J., 465
Wynn-Williams, N., 767

Yamada, H., 137
Yamada, T., 782
Yamamoto, H., 126
Yamamoto, T., 613
Yamamura, Y., 625
Yamashita, H., 150
Yanagisawa, K., 476
Yao, K. P., 605
Yasukawa, T., 766
Yen, H. C., 605
Yo, I., 768
Yokoyama, S., 137
Yokoyama, T., 766
Yoshida, K., 149
Yoshida, S., 312
Yoshimura, K., 783
Yoshinasu, M., 137
Yoshizawa, H., 477
Young, E. J., 766
Young, J. V. I., 775
Young, R. D., 767
Young, W. G., Jr., 151
Yu, P., 147
Yuasa, K., 126

Zaiman, E., 600
Zatzkin, H. R., 143
Zecca, A., 467
Zederfeldt, B., 611
Zeller, F., 290
Zetterberg, B., 297
Zimmerman, H., 148
Zorini, A. O., 776
Zubiani, M., 300
Zwi, S., 310

"1314." *See* Alpha-ethyl-thioisonicotinamide
Abdomen wall, in syndrome of congenital defects, 126
Abscess(es)
  pulmonary, 284
    in infant, massive hemoptysis from, causing emergency lung resection, 135
  tuberculous, epidural, simulating herniated lumbar intervertebral disk, 608
Absorbents, performance of, continuous flow, 609
Acetone-soluble lipids of tubercle bacilli, effect of, on antimicrobial activity of microphages, 626
Achalasia of cardia, carcinoma of esophagus, and hypertrophic pulmonary osteoarthropathy, 458
Acid(s)
  alpha-keto, isoniazid in metabolism of, 475
  ascorbic, intraleukocytic, in tuberculosis and silicotuberculosis, 133
  -base balance, trifluroethane in, 610
  blood lactic and pyruvic, and cardiorespiratory function, in chronic pulmonary tuberculosis, 309
  diaminopimelic, decarboxylase activity of, inhibited in *M. tuberculosis* by isoniazid, 311
  lactic, concentration of, in blood, before and after passing through lung, 478
  pantothenic, in vestibular nerve damage from streptomycin, 787
  sulfuric mist, respiratory response of guinea pigs to, 314
Acidosis
  hypoventilation, and hypoxia, in acute postoperative period, 469
  metabolic, of hyperventilation from controlled respiration, 789
Acriflavine in tubercle bacilli, 476
Adenovirus type 1 in viral "pneumonitis," 126
Adhesions, pleural, multiple, massive systemic pulmonary arterial shunt through, 604
Adjuvant, Freund's, and components of, effect on animal organs, 793
Adrenocorticosteroids. *See* Hormones
Aerosol(s)
  bacterial and other, syringe device for studies on, 151
  bronchodilator in bronchial asthma and pulmonary emphysema, 478
  carbachol and dust particles, inert, in human lungs, 309
  deposition in humans, 148
  sympathomimetic, inhalation of, lung-response changes after, 309
Africa
  Bantu of, tuberculosis, pulmonary, in, 468
  East, isoniazid and sulfone or PAS in pulmonary tuberculosis in, 785
  Kenya, prednisolone in chronic, drug-resistant, pulmonary tuberculosis, 620
  Rhodesia, Salisbury, incidence of pulmonary tuberculosis in, 782
  Union of South, tuberculin sensitivity, human and avian, in, 300
  Witwatersrand gold miners, respiratory function of, 310
Agammaglobulinemia and respiratory infections, 458
Air
  -borne droplet infection of tubercle bacilli in experimental animals, 150
  deposits and smoke in cancer and bronchitis mortality, 613
  "trapping," measurement of, in intrapulmonary gas distribution, 148
Airway resistance in ventilation and respiratory muscle activity, 310
Alkaline dust workers, respiratory function and disease in, 779
Allergy
  to food, bronchial asthma from, 766
  and influenza, Asian (1957), 287
  to tuberculin in geriatric patients, 133
Alpha-ethyl-thioisonicotinamide in tuberculosis, pulmonary, 620
Alpha-keto acids, isoniazid in metabolism of, 475
Aluminum worker, pulmonary fibrosis in, 779
Alveolar-capillary block syndrome and eosinophilia, pulmonary infiltration with, 129
Alveolous(i)
  carbon dioxide tension of, bedside measurement of, 147
  proteinosis of, pulmonary, 771
  ventilation of, and respiratory dead space in emphysema and silicosis, 148
Ambulatory treatment with pneumothorax, artificial, 289
Aminophylline in respiratory failure, 623
Amphotericin in moniliasis, generalized, 783
Amphotericin B
  intrathoracic injection of, in monilial empyema, 475
  in meningitis, cryptococcal, 144
  in mycoses, systemic, 303
Amyloid, tumor-like, of lower respiratory tract, 600
Anesthesia
  anomalies of ventilation during, 788
  ether, in the dog, hypocapnea in, 609
  nitrous oxide, in bronchoscopy, 775

pentobarbital sodium, respiration in guinea pigs under, 313
in thoracoplasty, 776
Aneurysm
aortic, syphilitic, penicillin in, 476
of artery
pulmonary, main, 458
pulmonary, segmental, and peripheral venous thrombosis, 458
pulmonary, arteriovenous, clinical description and therapy of, 766
Angiitis, generalized, prednisolone in, severe pulmonary involvement from, 126
Angiocardiography. *See* Roentgenography
Animals, domestic, public health aspect of tuberculosis in, 141
Antibody(ies)
antituberculous, in school children, 778
formation and bronchial asthma, in acquired hypogammaglobulinemia, 463
heterophile, in tuberculosis, pulmonary, 467
Antimicrobial(s)
activity of microphages, effect of acetone-soluble lipids of tubercle bacilli on, 626
combined, in staphylococcal infections, drug-resistant, 620
continuous, in bronchitis, chronic, in adults, 143
resistant strains of *M. tuberculosis* in patients not treated with, 303
in tuberculosis
of pancreas, 774
pulmonary, immediate and long-range results with, 304
Antimycobacterials, bacteriologic method for assessing, 783
Antistreptolysin titer in pulmonary tuberculosis, 772
Aorta
aneurysm of, syphilitic, penicillin in, 476
perforation of, after extrapleural plombage with synthetic balls, 775
stenosis of, calcific, surgery in adults, 295
thoracic, rupture of, in pulmonary tuberculosis, 290
Arteriosclerosis, pulmonary, primary, with rupture of pulmonary artery, 126
Artery(ies)
homotransplants of, in shunt operation in Fallot's tetralogy or tricuspid atresia, 611
pulmonary
causing obstructive emphysema in infants, 460
main, aneurysm of, 458
major, thrombosis of, 136
rupture of, in arteriosclerosis, primary, pulmonary, 126
segmental, aneurysms of, and peripheral venous thrombosis, 458
shunt of, massive, systemic, through multiple pleural adhesions, 604
Arthritis, rheumatoid, and lupus erythematosus, systemic, pleuropulmonary roentgenographic findings in, 619
Ascorbic acid. *See* Acid(s)
Asian influenza. *See* Influenza
Aspergillosis. *See* Mycoses
Aspergillus fumigatus in pulmonary disease, multiple, cavitary, 768
Asthma
bronchial
and antibody formation, in acquired hypogammaglobulinemia, 463
and bronchitis, chronic, hydrocortisone acetate inhalations in, 127
bronchodilator aerosol, new, in, 478
and chronic pulmonary emphysema, prednisolone in, 766
from food allergy, 766
hydrocortisone powder locally in, 127
pulmonary function screening tests in, 308
in children, physical conditioning program for, 284
Atelectasis
chronic, in pulmonary and bronchial blood flow, 477
congestive, fatal postoperative complication, 774
consolidation-, nonobstructive, after thoracotomy, 776
Australia, geriatric institution in, chronic bronchitis in, 766
8-Azaguanine in tubercle bacilli, 476

Bacillus(i)
acid-fast
atypical
in pulmonary disease, 791
in tuberculosis, pulmonary, 149
different species of, tuberculous diseases from, 624
from raw milk
biology of, 311
pathogenicity of, for guinea pigs, 791
drug-resistant, pulmonary tuberculosis from, treatment of, 787
Koch, in nontuberculous deaths, 289
nonacid-fast, in tuberculosis, pulmonary, 781
Bacterial flora of human tracheobronchial tree, 791
Bacterioides, 613
Balls, synthetic, in extrapleural plombage, aortic perforation after, 775
Bantu. *See* Africa
BCG
-induced immunity, isoniazid in, 305
infection with, in mouse resistance to bacterial endotoxin and *Salmonella enteritidis* infection, 794
and isoniazid, in tuberculosis prophylaxis, 617

isoniazid-resistant
immunogenicity of, in subcutaneous inoculation, 624
in vivo, 149
normal and isoniazid-resistant, in guinea pigs, 784
vaccination
in Brazil, 618
changes in cutaneous reaction with various doses of PPD-S after, 778
or chemotherapy in tuberculosis prevention, 617
conversion testing of schoolchildren after, 138
freeze-dried, in newborns, by multiple-puncture method, 782
in nonreactors in healthy leprosy contact children in Djakarta; correlation of lepromin and tuberculin reactions, 140
sensitivity of mice to endotoxin after, 313
tuberculous meningitis after, 140

Benzonatrate in dyspnea from chronic lung disease, 769

Biopsy
of lung
in "farmer's lung," 130
in pulmonary disease, disseminated, indeterminate, 136
pleural, Vim-Silverman needle in, 610
prescalene
lymph node, by Daniels' method, 136
node, 292
scalene node, in chest disease diagnosis, 469

Blood
flow, pulmonary and bronchial, chronic atelectasis in, 477
heat exchanger in hypothermia, controlled, studies in, 151
lactic
acid concentration in, before and after passing through lung, 478
and pyruvic acids, and cardiorespiratory function, in chronic pulmonary tuberculosis, 309
and leukocyte resistance in guinea pigs, isoniazid in, 306
menstrual, in tuberculosis of genital tract, diagnosis of, 134
vessel invasion in bronchogenic carcinoma, 469
volumes, modified Cr^{51} method of determination in older persons, 292

Boeck's sarcoid. *See also* Mycoses, sarcoidosis
causing pneumothorax, spontaneous, 127

Bone marrow and peripheral aspirates in bacteriologic diagnosis of childhood tuberculosis, 313

Branchial cysts, lateral, and tuberculosis, 290

Brazil
BCG vaccination in, 618
mass roentgenography in cities of, 615

Breathing aid, patient-cycled, in ventilatory efficiency in pulmonary emphysema and poliomyelitis, 478

British West Indies, Montserrat, tuberculin survey in, 616

Bronchiectasis
cystic, and pulmonary fibrosis, from intrathoracic goiter, 462
long-term treatment of, and chronic bronchitis, 784
in the Maori, 459
roentgenography in diagnosis of, 766
surgery in, 294
toruloma from, 606

Bronchitis
and cancer mortality, atmospheric deposit and smoke in, 613
chronic
in adults, antimicrobials in, 143
in Australian geriatric institution, 766
and bronchial
asthma, hydrocortisone acetate inhalations in, 127
carcinoma, 459
and bronchiectasis, long-term treatment of, 784
dust exposure related to disability and mortality from, 139
in industry, 613
laryngotracheo-, acute, 600

Bronchodilator aerosol in bronchial asthma and pulmonary emphysema, 478

Bronchography. *See* Roentgenography

Bronchomycosis fenisecarum, 614

Bronchopulmonary
ornithosis in France, 132
sequestration in infancy and childhood, 459

Bronchoscopy
and bronchography in bronchial cancer, 610
nitrous oxide anesthesia in, 775
and stratigraphy, in tuberculosis, bronchial, 302

Bronchospirometer (D.G.P. 56) for recording metabolism of both lungs, 146

Bronchotomography. *See* Roentgenography

Bronchus(i)
asthma in
and chronic pulmonary emphysema, prednisolone in, 766
from food allergy, 766
cancer of, bronchography and bronchoscopy in, 610
carcinoids of, biologic efficacy of extracts from, 793
carcinoma of
and chronic bronchitis, 459

pulmonary vessels in, electrokymography in, 128
carcinoma originating in, 284
blood vessel invasion in, 469
early diagnosis of, 601
hemoptysis heralding, 601
primary, regional lymph node metastasis in, 128
changes in, in pulmonary tuberculosis, endoscopic aspects of, 138
endo-, hamartochondroma of, 289
kinking of, after paravertebral thoracoplasty, causing giant cyst in case of tracheal lobe, 136
neoplasm of, primary, radiation therapy in, 143
and pulmonary blood flow, chronic atelectasis in, 477
pulmonary carcinoma originating from, and Cushing's syndrome, 284
rupture of, traumatic, 292
tree of, bronchotomography of, after lung resection, 142
tuberculosis of
bronchoscopy and stratigraphy in, 302
chemotherapy in, 772
steroids in, 133
Bullae, pulmonary, and infection, 459

Cadmium oxide fume, human exposure to, 778
Calcium
infusion test, intravenous, in sarcoidosis, 127
metastasis, so-called, 600
Canada, Quebec, Princeville, Q fever epidemic in, 298
Cancer. *See also* Carcinoma
bronchial
bronchography and bronchoscopy in, 610
pulmonary function in, 767
and bronchitis mortality, atmospheric deposit and smoke in, 613
incidence of, in Finland (1954), 296
isolation-perfusion techniques in, 775
of lung
death rates from, in England, Wales, and U.S.A., 139
and female pelvis, detection survey for, in Navajos, 778
metastatic, hypertrophic osteoarthropathy in, 465
mortality and cigarette ends' length, 614
and nose in nickle workers, 296
in women, 472
respiratory, in nickel workers, 296
Cancerigenesis, metal-, experiments in, 149
Carbon dioxide
elimination of, by lungs, effect of inhibition of carbonic anhydrase on, 477
gas, intravascular, in interatrial septal defects, 309
infrared analysis for, in respired gases with cyclopropane and ether, 623
tension, alveolar, bedside measurement of, 147
Carbonic anhydrase, inhibition of, effect on carbon dioxide elimination by lungs, 477
Carcinogens, disappearance of, from soot in human lungs, 778
Carcinoids, bronchial, biologic efficacy of extracts from, 793
Carcinoma. *See also* Cancer
bronchial
and chronic bronchitis, 459
pulmonary vessels in, electrokymography in, 128
bronchogenic, 284
blood vessel invasion in, 469
early diagnosis of, 601
hemoptysis heralding, 601
in lung cyst, 600
of lungs, and Cushing's syndrome, 284
primary, regional lymph node metastasis in, 128
and tuberculosis, pulmonary, simultaneous occurrence of, 772
chorio-, and related trophoblastic tumors in women, chemotherapy of, 128
of esophagus
achalasia of cardia, and hypertrophic pulmonary osteoarthropathy, 458
cellular detection of, 459
of lung
natural history of, 767
and tuberculosis, pulmonary, 607
primary, of lung, 284, 601
Cardiac. *See* Heart
Cardiopulmonary bypass
danger of hypoglycemia during, 775
temporary, in open heart surgery, 294
Cardiorespiratory
function and blood lactic and pyruvic acids, in chronic pulmonary tuberculosis, 309
syndrome of extreme obesity, 310
Cartilage, autogenous, in bifid sternum, 776
Catheterization of left heart by transbronchial route, 479
Cavity(ies)
open-healing, surgery in, 294
pulmonary, walls of, crescent-shaped cells in, 479
tuberculous
pulmonary, experimental production of, in dogs, 793
roentgenographic diagnosis of, limitations of, 783
streptomycin and isoniazid in, 306

Cell(s)
crescent-shaped, in walls of pulmonary cavities, 479
in detection of carcinoma of esophagus, 459
metaplastic, in sputum, in pulmonary eosinophilia, 626
Cerebrum, meningitis of, tuberculous spinal meningitis without, 608
Chemodectoma. *See* Tumor(s)
Chemotherapy
or BCG vaccination in tuberculosis prevention, 617
of choriocarcinoma and related trophoblastic tumors in women, 128
long-term, in tuberculosis, pulmonary, 145
in normal and isoniazid-resistant BCG in guinea pigs, 784
in tuberculoma-like shadows, 773
in tuberculosis
bronchial, 772
present status of, 619
pulmonary, relapse after, 784
Chest
diseases, diagnosis of, scalene node biopsy in, 469
murmur of, continuous, hypernephroma as, 131
pain in diagnosis of gastric ulcer, 606
shotgun-blast injuries of, close proximity, 135
supervoltage irradiation of, total, and intravenous infusion of stored autogenous marrow, 619
Chickenpox pneumonia, prednisolone in, 132
Chloroform, intoxication from, chronic, 285
Chylothorax in the newborn, 601
Cigarette(s), length of ends, and lung cancer mortality, 614
Ciliary streaming through larynx and trachea, 481
Circulation
collateral, between spleen transposited into thoracic cavity and the vena cava superior, 777
extracorporeal, and postoperative erythrocyte destruction, 611
and heart decompensation, emphysema in, 128
Coal
miners
pneumoconiosis, 140
mechanical properties of lung in, 779
workers from Cumberland, dust content of lungs of, 778
Coccidioidomycosis. *See* Mycoses
Collapse therapy and resection in pulmonary tuberculosis, relapses after, 612
Colon, transplant of, intrathoracic, in esophageal stricture from corrosives, 611
Cornea of mouse, *M. lepraemurium* in, 791
Cor pulmonale
acute, in schistosomiasis, acute, 605
chronic, cardiac arrhythmias in, 146
in tuberculosis, pulmonary, 773
Corticosteroids. *See* Hormones
Cough, fainting associated with, 622
"Cough" fracture of ribs, pneumothorax in, 767
Cr^{51} method, modified, in determination of blood volumes in older persons, 292
Group, predisposition to, 460
Cryptococcosis. *See* Mycoses
Culture, single-cell, of tubercle bacilli, 792
Cushing's syndrome and carcinoma, bronchogenic, of lungs, 284
Cyclopropane
epinephrine arrhythmias, suppression of, by phenothiazine, 291
and ether in respired gases, infrared analysis for carbon dioxide in, 623
Cycloserine
and isoniazid, diabetic signs caused by high doses of, in rats, 625
D-Cycloserine and isoniazid, effect of, on purified peroxidase and the peroxidase activity of *M. tuberculosis*, 784
neurotoxicity of, 621
and pyrazinamide in tuberculosis, pulmonary, 145
or pyrazinamide in animals vaccinated with inactivated tubercle bacilli, hemagglutinin and globulin behavior in, 313
in tuberculosis, 304
pulmonary, drug-resistant, 145
Cyst(s)
branchial, lateral, and tuberculosis, 290
bullous, isolated, of lung, 460
in disease of lung, 285
giant, from tracheal lobe, from kinking of bronchus after paravertebral thoracoplasty, 136
hydatid
hepatothoracic migrations of, 285
of liver, causing suprahepatic portal hypertension, 460
multiple, of lung, 460
of lung
bronchogenic carcinoma in, 600
congenital, malignant degeneration of, 128
mediastinal, thymic, 128

Daniels' method of prescalene lymph node biopsy, 136
Death. *See* Mortality
Decolorization resistance of mycobacteria, 792
Densiography. *See* Roentgenography
Diabetes in rats given large amounts of isoniazid and cycloserine, 625
Diaminopimelic acid. *See* Acid(s)
Diaphragm
endometriosis of, causing pneumothorax, 288
peritoneopericardial hernia of, 135
in syndrome of congenital defects, 126

Disk, intervertebral, lumbar, herniated, epidural tuberculous abscess simulating, 608
Drainage
intracavitary suction tube-, in staphylococcic tension pneumatoceles, 293
of pulmonary veins to right side of heart, surgery in, 295
Drugs
antimycotic, study of, by *Galleria mellonella* test, 303
antituberculous, and kanamycin, in experimental tuberculosis of mice and guinea pigs, 307
resistance to, real-, phenotypic-, and pseudo-, 303
-resistant
chronic, tuberculosis, pulmonary, in Kenya, prednisolone in, 620
tubercle bacilli
and postpneumonectomy complications, 787
tests for, 787
tuberculosis, pulmonary, treatment of, 787
trend in nonhospitalized tuberculous patients, 619
Ductus, artificial, temporary, in surgery in ventricular septal defects and severe pulmonary hypertension, 470
Dust
alkaline, workers in, respiratory function and disease in, 779
coal mine-, with low quartz content, silicotic lesions from, 480
content of lungs of Cumberland coal workers, 778
exposure to, bronchitis, chronic, disability and mortality from, 139
particles, inert, and carbachol aerosol, in human lungs, 309
quartz, pure, silicotic lesions from, 480
road, thorium in, inhalation of, on Niue Island, 473

Ear canal, anatomic variations pertaining to stethoscope earpiece fit, 772
Echinococcus disease in the U. S., 296
Effusion(s)
infrapulmonary, 143
malignant, prednisone in, 144
pericardial, massive, angiocardiography in surgery of, 142
pleural
solitary, from myxedema, 128
tuberculous
in children, transient rounded lesions after, 467
diagnosis of, 607
Electrocardiography, irregularities in, after surgery in pulmonary tuberculosis, 612
Electro-encephalography
in compression of superior vena cava during thoracotomy, 777
in cycloserine neurotoxicity, 621
transoperatory, 775
Electrokymography. *See* Roentgenography
Electrophoresis, paper, of serum proteins in pulmonary tuberculosis, 468
Emphysema
and health, oxygen consumption and respiratory muscles in, 790
obstructive, in infants, from anomalous course of left pulmonary artery, 460
prevalence and role of, in cardiac and circulatory decompensation, 128
pulmonary
bronchodilator aerosol, new, in, 478
chronic, and bronchial asthma, prednisolone in, 766
and peptic ulcer, 129
and poliomyelitis, patient-cycled breathing aid in, 478
regulation of pulmonary ventilation in, 478
and silicosis, respiratory dead space and alveolar ventilation in, 148
unilateral, and "vanishing lung," 604
Empyema
in children, 286, 461
monilial, intrathoracic injection of amphotericin B in, 475
staphylococcal, 129
in infants and children, surgery in, 470
tuberculous, pyrazinamide used topically in, 307
Endometriosis of diaphragm causing pneumothorax, 288
Endoscopy in tuberculosis, pulmonary, bronchial changes in, 138
Endothelioma *See* Tumor(s)
Endotoxin
bacterial, effect of BCG infection on resistance of mice to, 794
sensitivity of mice to, after vaccination with BCG, 313
Endotracheal aspirate of healthy newborns, 310
England
cancer of lung, death rates from, 139
Cumberland coal workers, dust content of lungs of, 778
R. A. F., acute respiratory disease in, 1955–1957, 141
Enteritis, tuberculous, perforated, acute, surgery in, 468
Enzymes of vitamin B_6, isoniazid and its derivatives in, 785
Eosinophilia
and alveolar-capillary block syndrome, pulmonary infiltration with, 129
in granuloma of lung, 130

pulmonary
metaplastic cells in sputum in, 626
tropical, 461
Epinephrine -cyclopropane arrhythmias, suppression of, by phenothiazine, 291
Erythema nodosum and sarcoidosis, 286
Erythrocyte(s)
postoperative destruction of, and extracorporeal circulation, 611
sedimentation rate in plasma detergent mixtures, 481
Esophagitis, reflux, severe, pathologic changes in, 767
Esophagotomy, sclerodermic, 466
Esophagus
carcinoma of, 458
cellular detection of, 459
foreign body in, acquired esophagotracheal fistula secondary to, 470
leiomyoma of, 772
stricture of, from corrosives, intrathoracic colon transplant in, 611
ulcer of, 606
Ether
anesthesia in the dog, hypocapnea in, 609
and cyclopropane in respired gases, infrared analysis for carbon dioxide in, 623
Ethionamide in tuberculosis, 788
Expiratory volume per second, maximal, and normal vital capacity values, 311

Fainting associated with coughing, 622
Fallot's tetralogy, shunt operation with arterial homotransplants in, 611
"Farmer's lung." *See* Mycoses
Fat, neutral, intrapulmonary, studies on, 769
Fibrocystic disease of pancreas, roentgenography in, 286
Fibrosis(es)
mediastinal, idiopathic, 461
pulmonary
in aluminum worker, 779
interstitial, diffuse, primary, 131
steroids in, 462
Fibrothorax, right heart and lesser circulation function in, 479
Finland
cancer incidence in, (1954), 296
influenza mortality in (1957), 140
intradermic tuberculin test in, 616
Fistula(s)
bronchopleural, postoperative, and preoperative bronchography, 782
esophagotracheal, acquired, secondary to foreign body in esophagus, 470
Flora, bacterial, of human tracheobronchial tree, 791
Fluoroscopy. *See* Roentgenography
Fluothane®. *See* Trifluroethane
Food allergy, bronchial asthma from, 766
Fracture, "cough," of ribs, pneumothorax in, 767
France
Armed Forces of, in Germany, diagnosis of tuberculosis in, 467
Northern, bronchopulmonary ornithosis in, 132
Freund's adjuvant and components of, effect on animal organs, 793
Fuchsin -malachite green staining of tubercle bacilli, 149
Funnel chest, operative technique in, 611

Galleria mellonella test, in antimycotic drug study, 303
"Galloping phthisis," 607
Gas(es)
carbon dioxide, intravascular, in interatrial septal defects, 309
distribution, intrapulmonary, air "trapping" measurement in, 148
respired, cyclopropane and ether in, infrared analysis for carbon dioxide in, 623
Gasoline poisoning, acute, 602
Gel, immuno-precipitation of polysaccharides of tubercle bacilli in, 792
Genital tract, tuberculosis of, menstrual blood in diagnosis of, 134
Genitourinary tuberculosis, infectiousness of, in women, 291
Geriatrics, Australian institution for, chronic bronchitis in, 766
Glass
adsorption of tuberculin to, 301
microspheres as inhalation tracers, 151
Globulins and hemagglutinins in animals vaccinated with inactivated tubercle bacilli and treated with cycloserine or pyrazinamide, 313
Goiter
intrathoracic, causing bronchiectatic, destroyed lobe, 462
and myxedema during PAS treatment of pulmonary tuberculosis, 145
Gold miners in Witwatersrand, respiratory function of, 310
Gonads, reducing radiation to, in pediatric diagnosis, 474
Granuloma. *See* Tumor(s)
Granulomatosis, Wegener's, 133

Hamartochondroma. *See* Tumor(s)
Hamman-Rich syndrome, 131
differential diagnostic problems of, 768
Heart
achalasia of, 458
arrhythmias in cor pulmonale, chronic, 146
bypass without artificial oxygenation, 146

and circulatory decompensation, emphysema in, 128
congestive failure of, effect of body position change on lung compliance in, 789
disease
congenital
in infants, pulmonary complications of, 129
pulmonary hypertension and vascular change in, 623
pediatric, comparison of 3 diagnostic methods in, 781
pulmonary, anoxemic, 308
and tuberculosis, mass surveys, 141
left, catheterization by transbronchial route, 479
open, surgery of, temporary cardiopulmonary bypass in, 294
pathologic findings in, in death from chronic pulmonary tuberculosis, 482
right, and function of lesser circulation in fibrothorax, 479
in syndrome of congenital defects, 126
Hemagglutinins and globulins in animals vaccinated with inactivated tubercle bacilli and treated with cycloserine or pyrazinamide, 313
Hemangioma. *See* Tumor(s)
Hemodynamics
of pulmonary stenosis without ventricular septal defect, 623
in tricuspid stenosis, 790
Hemopneumothorax, spontaneous, massive, emergency thoracotomy for, 777
Hemoptysis
heralding bronchogenic carcinoma, 601
massive, in infant with lung abscess, 135
Hemosiderosis
pulmonary
idiopathic, 462
in adults and adolescents, 462
nonrecurrent, early, in an adult, 130
prednisolone in, 768
Heparin in intravascular aggregation in induced hypothermia, 611
Hernia
diaphragmatic
peritoneopericardial, 135
spontaneous strangulation in, 470
of lumbar intervertebral disk, epidural tuberculous abscess simulating, 608
Herniorrhaphy, hiatal, right-sided approach in, 136
Hexamethonium therapy and pulmonary changes, 602
Histoplasmin reaction, cutaneous, in Campania, 297
Histoplasmosis. *See* Mycoses
Hodgkin's disease
and pregnancy, 286
prognosis of, 602
and torulosis, 288
Hormones
adrenocortical, in miliary tuberculosis, 144
adrenocorticosteroids, in tuberculosis, 602
corticosteroids
in experimental tuberculosis in guinea pigs, 794
in tuberculous inflammation, 482
hydrocortisone
acetate inhalations in bronchial asthma and chronic bronchitis, 127
in intracellular growth of tubercle bacilli, 793
powder in bronchial asthma, 127
prednisolone
in angiitis, generalized, severe pulmonary involvement from, 126
in bronchial asthma and chronic pulmonary emphysema, 766
in chickenpox pneumonia, 132
in pulmonary hemosiderosis, idiopathic, 768
in tuberculosis, pulmonary, chronically drug-resistant, in Kenya, 620
prednisone
in effusions, malignant, 144
in sarcoidosis, 465
steroids
in hypercalcemia and renal insufficiency in sarcoidosis, 620
in pulmonary fibroses, 462
in tuberculosis, bronchial, 133
Hospital(s)
chest, respiratory function testing in, 789
general, tuberculosis treatment in, 142
Sea View, principal causes of death at, 472
staphylococcal infections in, 615
tuberculosis, in Brazil, 473
Hungary, childhood tuberculosis in, 301
Hydrocortisone. *See* Hormones
Hygroma. *See* Tumor(s)
Hypercalcemia and renal insufficiency in sarcoidosis, steroids in, 620
Hypernephroma. *See* Tumor(s)
Hypertension
portal, suprahepatic, from hydatid cyst of liver 460
pulmonary
monolobar, acute, experimental, 788
primary, 147
severe, and ventricular septal defects, temporary artificial ductus in surgery of, 470
and vascular change, in congenital heart disease, 623
venous, pulmonary, diseases with, causing histologic changes in lung, 462
Hyperventilation from controlled respiration, metabolic acidosis of, 789
Hypocapnea in ether anesthesia in the dog, 609
Hypogammaglobulinemia, acquired, antibody formation in, in bronchial asthma, 463

Hypoglycemia, danger of, during cardiopulmonary bypass, 775
Hypothermia
controlled, with blood heat exchanger, 151
immersion, alterations in pulmonary and peripheral vascular resistance in, 794
induced, heparin in intravascular aggregation in, 611
Hypoventilation
in acute postoperative period, 469
in obesity, 789
Hypoxia in acute postoperative period, 469

Iconography. *See* Roentgenography
Icterus and fever from isoniazid, 785
Indonesia, Djakarta, lepromin and tuberculin reactions correlated in, 140
Industry
another pneumoconiosis in, 298
bronchitis, chronic, in, 613
Infarction, pulmonary, after segmental resection, 471
Influenza
Asian (1957) in allergics, 287
fatal
clinicopathologic study of, 768
respiratory tract and lungs in, 463
pneumonia in, 287
epidemic
of 1957, pneumonia in adults during, roentgenography in, 602
of 1957–1958, in Sweden, vaccination trials, 297
mortality in Finland (1957), 140
neurologic and hepatic disorders in, 463
pandemic, from virus type A/Asia/57, in Norway, 297
pandemic of 1957–1958
epidemiologic, clinical, and serologic studies of intrahospital epidemic of, and vaccination, 603
isolation of Asian strain virus from patients with pulmonary complications, 603
pulmonary complications in, 603
Infrared analysis for carbon dioxide in respired gases with cyclopropane and ether, 623
Inhalation tracers, glass microspheres as, 151
Intestine, small, sarcoidosis of, 134
Intracavitary suction tube drainage in staphylococcic tension pneumatoceles, 293
Iodide therapy and unusual electrolyte disturbance in pulmonary aspergillosis with cavitation, 604
Iproniazid causing jaundice, pathology of, 304
Irradiation. *See* Radiation
Isolation-perfusion techniques in cancer, 775
Isoniazid
and BCG in tuberculosis prophylaxis, 617
in BCG-induced immunity, 305
in blood and leukocyte resistance in guinea pigs, 306
and cycloserine, diabetic signs caused by high doses of, in rats, 625
and D-cycloserine, effect of, on purified peroxidase and the peroxidase activity of *M. tuberculosis*, 784
and derivatives
metabolism of, perfusion experiments in liver, 621
in vitamin B_6 enzymes (tryptophanase), 785
free
assay of, in serum, vertical diffusion test in, 476
serum levels of, maximal, in resistant strains, 305
glucuronate of, in tuberculosis, pulmonary, 785
icterus and fever from, 785
inactivation in man, 621
inhibiting diaminopimelic acid decarboxylase activity in *M. tuberculosis*, 311
kanamycin, and streptomycin in *M. tuberculosis* in mouse, 626
in lymph nodes of guinea pigs, reactivation of tuberculous infection in, 305
in metabolism of alpha-keto acids, 475
-pyrazinamide in tuberculosis, pulmonary, 622, 786
and pyridoxine, effect on serum inhibitory power, 146
radioactive, absorbance of, by "free" and "protected" microorganisms, 480
and rehabilitation, in tuberculosis, skeletal, 621
resistance
peroxidase reaction in tubercle bacilli in, 792
and streptomycin resistance, 306
in tuberculosis, pulmonary, primary, nontreated, 300
-resistant
BCG
immunogenicity of, in subcutaneous inoculation, 624
in vivo, 149
and normal BCG in guinea pigs, chemotherapy in, 784
and streptomycin-resistant tubercle bacilli, virulence of, 787
tubercle bacilli, peroxidase reaction in population analysis of, 312
serum level of, and *M. tuberculosis* resistance, vertical diffusion test in, 306
streptomycin-, and PAS-resistant tubercle bacilli, 792
streptomycin, and PAS in tubercle bacilli in cells from two different sources, 793
and streptomycin in tuberculous cavities, 306
-streptovaricin in tuberculosis, experimental, of guinea pigs, 144
and sulfone or PAS in tuberculosis, pulmonary, in East Africans, 785

therapy, resistant strains from, maximal serum levels of free isoniazid in, 305
venous perfusion of, and water-soluble thiosemicarbazone, in pulmonary tuberculosis, 306
Isonicotinic acid hyrazide. *See* Isoniazid
Israel, Haifa, myocarditis, interstitial, primary, in, roentgenography in, 474
Italy, Campania, histoplasmin reaction, cutaneous, in, 297

Japan
mycotic infections in, 605
tuberculosis in, current status of, 618
Jaundice from iproniazid, pathology of, 304
Johne's disease, doses of *M. johnei* for production of, in cattle, 791

Kanamycin
antituberculous activity of, *in vitro* studies on, 307
and other drugs, in experimental tuberculosis of mice and guinea pigs, 307
streptomycin, acriflavine, 8-azaguanine, and Na azide, in tubercle bacilli, 476
susceptibility
of tubercle bacilli, 149
determination of, by direct and indirect methods, 307
in tuberculosis
experimental, of mice, 626
pulmonary, 786
in vitro, and in human tuberculosis, 476
Kartagener syndrome 603
Keratoconjunctivitis, phlyctenular, recurrent, desensitization to tuberculin in, 773
Kidney insufficiency and hypercalcemia in sarcoidosis, steroids in, 620
Koch bacillus in nontuberculous deaths, 289
Kveim tests in sarcoidosis and family contacts, 473

Lactic acid. *See* Acid(s)
Laparatomy, high, lung volume and function studies after, 292
Laryngitis, tuberculous, in sanatorium patients, 135
Laryngotracheobronchitis
acute, 600
0.125% superinone in, 131
Larynx
ciliary streaming through, 481
palsy of, after pneumothorax, spontaneous, 132
tuberculosis of, 609
Leiomyoma. *See* Tumor(s)
Leprology, irradiated tuberculosis vaccine in, 479
Lepromin and tuberculin reactions, correlation between, and influence of BCG on nonreactors in leprosy contact children in Djakarta, 140
Lesion(s)
"coin," hemangiomas, sclerosing, circumscribed, of lung, appearing as, 466
metastatic, pulmonary resections for, 136
pulmonary
metastatic, surgery in, 612
and rheumatoid disease, 133
thrombo-embolic, 131
rounded, transient, after tuberculous pleural effusion in children, 467
silicotic, from pure quartz dust, 480
Leukemoid reaction in hematogenous tuberculosis, 774
Leukocyte and blood resistance in guinea pigs, isoniazid in, 306
Lipids, acetone-soluble, of tubercle bacilli, in antimicrobial activity of microphages, 626
Liver
hydatid cyst of, causing suprahepatic portal hypertension, 460
and neurologic disorders in influenza, 463
perfusion experiments in isoniazid metabolism, 621
Lobe(s)
bronchiectatic, destroyed, from intrathoracic goiter, 462
middle, syndrome of, 288
tracheal, cyst from, from kinking of bronchus after paravertebral thoracoplasty, 136
Lobectomy in tuberculosis in children, 137
Lobeline as a smoking deterrent, 771
Löffler's syndrome from penicillin, intratracheal, 786
Löwenstein-Jensen medium. *See* Medium(a)
Lung(s)
abscesses of, 284
agenesis of, 130
alveolar proteinosis of, 771
aneurysm of, arteriovenous, 766
angiography in, in tuberculosis, roentgenography in urinary tract after, 474
arterial shunt of, massive, systemic, through multiple pleural adhesions, 604
arteriosclerosis of, primary, with rupture of pulmonary artery, 126
artery of
left, causing obstructive emphysema in infants, 460
main, aneurysm of, 458
major, thrombosis of, 136
pulmonary, segmental, and peripheral venous thrombosis, 458
rupture of, in arteriosclerosis, pulmonary, primary, 126
aspergillosis of, with cavitation, iodide and electrolyte disturbance in, 604
atrophy of, idiopathic, 604
and bronchial blood flow, chronic atelectasis in, 477

bullae of, and infection, 459
cancer
death rates
in England and Wales and U.S.A., 139
detection survey for, in Navajos, 778
mortality
and cigarette ends' length, 614
in nickel workers, 296
in women, 472
carbon dioxide elimination by, effect of inhibition of carbonic anhydrase on, 477
carcinoma
bronchogenic, and Cushing's syndrome, 284
natural history of, 767
primary, 284, 601
and tuberculosis, pulmonary, 607
cavity walls of crescent-shaped cells in, 479
changes in, and hexamethonium therapy, 602
circulation of, after pneumothorax and vagotomy in dogs, 478
compliance in congestive heart failure, effect of body position change on, 789
complications
in heart disease, congenital, in infants, 129
in influenza pandemic of 1957–1958, 603
in cryptococcosis, 285
cyst
bronchogenic carcinoma in, 600
bullous, isolated, 460
congenital, malignant degeneration of, 128
hydatid, multiple, 460
cystic disease of, 285
densiography of, fluoroscopic, 619
disease
atypical acid-fast bacilli in, 791
cavitary, multiple, *Aspergillus fumigatus* in, 768
chronic, benzonatrate in, 769
indeterminate, disseminated, lung biopsy in, 136
miliary, roentgenography in, 142
pre-existing, in lung trauma, 604
of dog, stretch reflexes from, to the systemic circulation, 481
dust content of, in Cumberland coal workers, 778
778
effects of inert dust particles and carbachol aerosol on, 309
elastic recoil of, manual versus automatic ventilation in, 310
emphysema of
chronic, and bronchial asthma, prednisolone in, 766
and peptic ulcer, 129
eosinophilia
and alveolar-capillary block, syndrome infiltrating, 129
metaplastic cells in sputum in, 626
tropical, 461
excised, inflationary force from pulmonary vascular distention in, 791
"farmer's," biopsy report on, 130
fibrosis
in aluminum worker, 779
interstitial, diffuse, primary, 131
steroids in, 462
foreign body in, 137
function of
in cancer, bronchial, 767
effects of chest radiation on, 794
after pneumonectomy for tuberculosis, pulmonary, 790
screening tests in bronchial asthma, 308
granuloma of, eosinophilic, 130
hemangiomas of, sclerosing, circumscribed, appearing as "coin" lesions, 466
hemosiderosis of
idiopathic, 462
in adults and adolescents, 462
nonrecurrent, early, in an adult, 130
prednisolone in, 768
histologic changes in, in diseases with pulmonary venous hypertension, 462
histoplasmosis of, 770
human, disappearance of carcinogens from soot in, 778
hypertension of
monolobar, acute, experimental, 788
primary, 147
severe, and ventricular septal defects, temporary artificial ductus in surgery of, 470
and vascular change, in congenital heart disease, 623
infarction of, after segmental resection, 471
infections, in childhood, surgery in, 295
infiltration of, chronic granulomatous disease in, 475
involvement, severe, of, in generalized angiitis, after prednisolone, 126
lesions
metastatic, surgery in, 612
and rheumatoid disease, 133
thrombo-embolic, 131
mechanical properties of, in coal miners' pneumoconiosis, 779
megakaryocytes of, increased, in postoperative thromboembolism, 612
metabolism of, bronchospirometer (D.G.P. 56) for recording of, 146
neoplasms, benign, 466
nodules, metastatic, excavation of, roentgenography in, 475
ossification of, nodular, disseminated, in mitral stenosis, 769
osteoarthropathy of, hypertrophic, 458

pathology in nickel workers, 297
resection
bronchotomography of bronchial tree after, 142
emergency, in massive hemoptysis in infant with lung abscess, 135
for metastatic lesions, 136
in tuberculosis, 472
in children, 138
and respiratory tract in Asian influenza, fatal, 463
resistance, alterations in, in immersion hypothermia, 794
sarcoidosis of, roentgenography in, 143
sarcoma of, successful surgery in, 613
scleroderma of, 604
segments, selective angiography in, 474
silicosis of, in retired miners, 781
-staphylococcal infection in adults, 288
stenosis of, without ventricular septal defect, 623
and stomach, plasmacytoma of, 467
trauma, pre-existing pulmonary disease in, 604
tumor, malignant, presenting as cystic formation, 128
"vanishing," and unilateral emphysema, 604
vascular distention of, in excised lungs, inflationary force from, 791
veins of
drainage of, to right side of heart, surgery in, 295
partial anomalous drainage of, 137
ventilation, regulation of, in pulmonary emphysema, 478
vessels in bronchial carcinoma, electrokymography in, 128
volume and function, postoperative studies of, 292
Lupus erythematosus
systemic
pleuropulmonary symptoms of, 464
and rheumatoid arthritis, pleuropulmonary roentgenographic findings in, 619
Lymph node(s)
of guinea pigs treated with isoniazid, reactivation of tuberculous infection in, 305
intrathoracic, giant, 769
Koch bacillus in, in nontuberculous deaths, 289
metastasis of, regional, in carcinoma, bronchogenic, primary, 128
prescalene, biopsy of, by Daniels' method, 136

Malachite green -fuchsin staining of tubercle bacilli, 149
Maori. *See* New Zealand
Marrow, autogenous, stored, intravenous infusion of, after total thoracic supervoltage irradiation, 619
Mass surveys. *See* Roentgenography
Mediastinum
borders of, roentgenography in, 302
chemodectoma of, 289
cyst of, thymic, 128
fibrosis of, idiopathic, 461
Medium(a)
culture, with multiple agents, in tests for drug-resistant tubercle bacilli, 787
Dubos Tween®-albumin, nicotinic acid produced by mycobacteria cultured on, 312
liquid, synthetic, nicotinic acid produced by mycobacteria cultured on, 312
Löwenstein-Jensen, freeze-dried, in culture of *M. tuberculosis*, 624
solid, nicotinic acid produced by mycobacteria cultured on, 312
Megakaryocytes, pulmonary, increased, in postoperative thromboembolism, 612
Meningitis
cryptococcal, amphotericin B in, 144
spinal, tuberculous, without cerebral meningitis, 608
tuberculous, 608
in the adult, 468
after BCG vaccination, 140
in mental deficiency, 290
Meningocele
intrathoracic, 769
lateral, 464
Mental deficiency, tuberculous meningitis in, 290
Mepazine, pharmacodynamic control of myocardial irritability by, in dogs, 314
Mesothelioma. *See* Tumor(s)
Metal cancerigenesis, experiments in, 149
Metastasis
calcium, so-called, 600
of lymph nodes, regional, in carcinoma, bronchogenic, primary, 128
Microorganisms, "free," and "protected," absorbance of radioactive isoniazid by, 480
Microphages, antimicrobial activity of, effect of acetone-soluble lipids of tubercle bacilli on, 626
Milk
raw
acid-fast bacilli from
biology of, 311
pathogenicity of, for guinea pigs, 791
Miners
gold, in Witwatersrand, respiratory function of, 310
retired, progress of simple pulmonary silicosis in, 781
Moniliasis. *See* Mycoses
Mononucleosis, infectious, acute pericarditis as initial sign of, 770

Mortality
occupational, in 3 Swedish professions, 614
at Sea View Hospital, principal causes of, 472
Mycobacteria
protoplast formation from, by mycobacteriophage, 311
resistance to decolorization, 792
tuberculous, cytochemical reactions and catalase activity of, 479
unidentified, 624
Mycobacteriophage forming protoplasts from mycobacteria, 311
Mycobacterium
johnei in production of Johne's disease in cattle, 791
lepraemurium in mouse cornea, 791
tuberculosis
BCG infection with, in mouse resistance to bacterial endotoxin and *Salmonella enteritidis* infection, 794
culture method with freeze-dried Löwenstein-Jensen medium, 625
isoniazid inhibiting diaminopimelic acid decarboxylase activity in, 311
metabolic activity of, influence of age on, 311
in mouse tissue, kanamycin, isoniazid, and streptomycin in, 626
peroxidase activity of, effect of isoniazid and D-cycloserine on, 784
resistant strains in nontreated patients, 303
and serum isoniazid level, vertical diffusion test in determining resistance of, 306
virulent and nonvirulent strain of, trichloroacetic extracts of, 480
Mycoses
anti-, drugs, study of, by *Galleria mellonella* test, 303
aspergillosis
primary, allergic, and Schönlein-Henoch purpura, 604
pulmonary, with cavitation, iodide, and electrolyte disturbance in, 604
coccidioidomycosis, fatal, morphology and racial distribution of, 614
cryptococcosis. *See also* Mycoses, torulosis
cryptococcosis, 464
bronchiectatic, 606
of central nervous system, 144
with pulmonary involvement, 285
"Farmer's lung" (or bronchomycosis feniscarum), 614
biopsy report on, 130
histoplasmosis
generalized, fatal, 605
intrathoracic, roentgenography in, 474
microepidemic of, 781
in New Zealand, 464
and pericardial calcification, 770
pulmonary, 770
test, simplified, using precipitin-in-gel reactions, 139
infections with, in Japan, 605
moniliasis
bronchopulmonary, 770
generalized, amphotericin in, 783
sarcoidosis. *See also* Boeck's sarcoid
sarcoidosis, 465
clinical studies of, 127
and erythema nodosum, 286
and family contacts, Kveim tests in, 473
hypercalcemia and renal insufficiency in, steroids in, 620
of small intestine, 134
intravenous calcium infusion test in, 127
mass roentgenography in, 473
of nervous system, 605
prednisone in, 465
pulmonary, roentgenography in, 143
thoracic, in a Maori, 465
tubercles of, at site of tuberculin injections, 291
systemic, amphotericin B in, 303
torulosis. *See also* Mycoses, cryptococcosis and Hodgkin's disease, 288
Myocarditis, interstitial, primary, in Haifa, roentgenography in, 474
Myocardium, irritability of, pharmacodynamic control of, by mepazine in dogs, 314
Myxedema
and goiter during PAS treatment of tuberculosis, pulmonary, 145
in pleural effusion, solitary, 128

Na azide in tubercle bacilli, 476
Navajos, cancer of lung and female pelvis detection survey in, 778
Neoplasm. *See* Tumor(s)
Nerve(s)
disorders of, in influenza, 463
vestibular, damage of, from streptomycin, pantothenic acid in, 787
Nervous system
central, cryptococcosis of, 144
sarcoidosis of, 605
Netherlands, Amsterdam, roentgenographic survey, chest, second, of population of, 141
New Zealand
histoplasmosis in, 464
Maori
bronchiectasis in, 459
thoracic sarcoidosis in, 465
Niue Island, inhalation of thorium in road dust on, 473
Niacin test in differentiation of human tubercle bacilli, 311

Nickel workers
cancer of lung and nose in, 296
lung pathology in, 297
respiratory cancer in, 296
Nicotinic acid produced by mycobacteria cultured on synthetic liquid-, solid-, and Dubos Tween®-albumin media, 312
Nikethamide in respiratory failure, 623
Nitrous oxide anesthesia in bronchoscopy, 775
Nodes, scalene, biopsy, in chest disease diagnosis, 469
Nodules, metastatic, in lung, excavation of, roentgenography in, 475
Norway
influenza pandemic from virus type A/Asia/57 in, 297
Oslo, city hospitals in, prognosis of tuberculous pleurisy in, 615
Nose and lung cancer in nickel workers, 296

Obesity
extreme, cardiorespiratory syndrome of, 310
hypoventilation in, 789
Operation. *See also* Surgery
Operation, intrathoracic, and high laparotomy, lung volume and function studies after, 292
Ornithosis, bronchopulmonary, in France, 132
Orthovoltage rotation therapy, 143
Osteoarthropathy
hypertrophic, in cancer, metastatic, of lung, 465
pulmonary, 458
Ovarian tuberculosis, disseminated, acute, unilateral, 609
Oximetry, transoperatory, 471
Oxygen consumption and respiratory muscles in health and emphysema, 790
Oxygenation, artificial, cardiac bypass without, 146

Pacatal®. *See* Mepazine
Palsy, laryngeal, after pneumothorax, spontaneous, 132
Pancreas
fibrocystic disease of, roentgenography in, 286
tuberculosis of, antimicrobials in, 774
Pantothenic acid. *See* Acid(s)
Para-aminosalicylic acid
-resistant tubercle bacilli, 792
streptomycin, and isoniazid in tubercle bacilli in cells from two different sources, 793
or sulfone and isoniazid in tuberculosis, pulmonary, in East Africans, 785
in tuberculosis, pulmonary, goiter and myxedema from, 145
Paraffin, liquid, oil aspiration pneumonia from, 465
Paraplegia of lower extremities in intrathoracic dumbbell-shaped tumor, 606
Pelvis, female, cancer of, detection survey for, in Navajos, 778
Penicillin
in aneurysm, aortic, syphilitic, 476
intratracheal, Löffler's syndrome from, 786
phenoxymethyl, oral, in pneumococcal pneumonia, 622
Penicillin G and V, oral, 622
Penicillin V in respiratory disease, upper, acute, 476
Pentobarbital sodium anesthesia. *See* Anesthesia
Pericarditis, acute, in infectious mononucleosis, 770
Pericardium
calcification of, and histoplasmosis, 770
effusions of, massive, angiocardiography in surgery of, 142
in syndrome of congenital defects, 126
Peritoneopericardial diaphragmatic hernia, 135
Peroxidase
purified, effect of isoniazid and D-cycloserine in, 784
reaction
in population analysis of isoniazid-resistant tubercle bacilli, 312
in tubercle bacilli in isoniazid-resistance study, 792
Phenazine, derivative of, in bacteriologic and animal experiments, 307
Phenothiazine derivatives suppressing cyclopropane-epinephrine arrhythmias, 291
Phthisis, "galloping," 607
Plasma detergent mixtures, erythrocyte sedimentation rate in, 481
Plasmacytoma. *See* Tumor(s)
Pleura(e)
adhesions of, multiple, massive systemic pulmonary arterial shunt through, 604
biopsy of, Vim-Silverman needle in, 610
effusion of, solitary, from myxedema, 128
tuberculous
in children, transient rounded lesions after, 467
diagnosis of, 607
endothelioma of, primary, 606
Pleural fluid, protein concentration in, 132
Pleurisy, tuberculous, prognosis of, in Oslo city hospitals, 615
Pleuropulmonary symptoms of systemic lupus erythematosus, 464
Plombage, extrapleural, with synthetic balls, aortic perforation after, 775
Pneumatoceles, tension, staphylococcal intracavitary suction tube drainage in, 293
Pneumoconiosis, 615
coal miners', 140
mechanical properties of lung in, 779
industrial, another, 298
status of, 140

Pneumocystis carinii
pneumonia, 606
subclinical, 771
Pneumonectomy
post-, complications, and drug-resistant tubercle bacilli, 787
in tuberculosis, pulmonary, pulmonary function after, 790
Pneumonia
adenovirus, 465
in adults, during influenza epidemic of 1957, roentgenography in, 602
chickenpox, prednisolone in, 132
chronic, radiation therapy in, 302
in influenza, Asian, 287
influenzal
fulminating, 287
in stenosis, mitral, 287
interstitial, pathologic concept of, 465
oil aspiration, from liquid paraffin, 465
plasma-cell, interstitial, 465
pneumococcal, phenoxymethyl penicillin, oral, in, 622
Pneumocystis carinii, 606
from *Staphylococcus aureus*, 771
Pneumonitis
Pneumocystis, subclinical, 771
radiation-induced, 783
rheumatic
clinical and laboratory findings, 466
pathology of, 466
viral, fatal, isolation of adenovirus type 1 from, 126
Pneumoperitoneum in pulmonary tuberculosis, 776
Pneumothorax
artificial, ambulatory treatment with, 289
in "cough" fracture of ribs, 767
intrapleural, in pulmonary tuberculosis, 776
spontaneous
Boeck's sarcoid causing, 127
laryngeal palsy after, 132
recurring, chronic, from endometriosis of diaphragm, 288
and vagotomy in dogs, pulmonary circulation after, 478
Polaroid Land process in roentgenographic transparencies for projection, 475
Poliomyelitis, patient-cycled breathing aid in, 478
Polysaccharides of tubercle bacilli, immuno-precipitation of, in gel, 792
Porocephalosis, nymphal, 131
Porphyria, intermittent, acute, with respiratory paralysis, 288
Pott's disease, 134
PPD. *See* Tuberculin
Prednisolone. *See* Hormones
Prednisone. *See* Hormones
Pregnancy and Hodgkin's disease, 286
Prescalene node biopsy, 292
Prophylaxis in tuberculosis, 144
Protein(s)
concentration of, in pleural fluid, 132
serum, paper electrophoresis of, in pulmonary tuberculosis, 468
Proteinemia in tuberculosis, pulmonary, 290
Proteinosis, alveolar, pulmonary, 771
Protoplast(s) from mycobacteria, formation of, by mycobacteriophage, 311
Pulmonary. *See also* Lung(s)
Pulmonary heart disease, anoxemic, 308
Purpura, Schönlein-Henoch, and aspergillosis, primary, allergic, 604
Pyrazinamide
and cycloserine, in tuberculosis, pulmonary, 145
or cycloserine, in animals vaccinated with inactivated tubercle bacilli, hemagglutinin and globulin behavior in, 313
and isoniazid in tuberculosis, pulmonary, 622, 786
used topically in tuberculous empyema, 307
in tuberculosis, pulmonary, 477
drug-resistant, 145
Pyridoxine and isoniazid, effect on serum inhibitory power, 146

Q fever epidemic in Princeville, Quebec, 298
Quartz dust. *See* Dust(s)

Radiation
chest, effects on pulmonary function, 794
diagnostic and therapeutic, in an urban population, 474
to gonads, reduction of, in pediatric diagnosis, 474
-induced pneumonitis, 783
supervoltage, thoracic, total, and intravenous infusion of stored autogenous marrow, 619
therapy
in neoplasm, bronchial, primary, 143
in pneumonia, chronic, 302
Radiography. *See* Roentgenography
Radiology. *See* Roentgenography
Rehabilitation
professional and social, in tuberculosis, pulmonary, 301
in tuberculosis, skeletal, 621
Relapse
after surgery for pulmonary tuberculosis, 612
of tuberculosis, pulmonary, after completion of chemotherapy in, 784
Resection
and collapse therapy, in tuberculosis, pulmonary, relapses after, 612
in mesothelioma, multiple, 612
pulmonary

bronchotomography of bronchial tree after, 142
in massive hemoptysis in infant with lung abscess, 135
for metastatic lesions, 136
in tuberculosis, 472
in children, 138
segmental, pulmonary infarction after, 471
in tuberculosis, pulmonary, 472, 612
Respiration
controlled
hyperventilation from, metabolic acidosis of, 789
in status epilepticus, 622
factors in, during heavy exercise, 310
in guinea pigs under pentobarbital sodium anesthesia, 313
Respiratory
dead space, and alveolar ventilation, in emphysema and silicosis, 148
disease
acute, in R. A. F., 1955–1957, 141
in childhood, hemadsorption viruses in, 618
staphylococcal infection in, 606
upper, acute, penicillin V in, 476
effort, priority of, over respiratory center, in regulation of pulmonary ventilation in pulmonary emphysema, 478
failure, aminophylline, nikethamide, and sodium salicylate in, 623
function
and disease in alkaline dust workers, 779
in the retractive syndromes, 479
tests, in chest hospital work, 789
in tuberculosis, pulmonary, chronic, 790
of Witwatersrand gold miners, 310
infections, and agammaglobulinemia, 458
muscle activity, and ventilation, airway resistance in, 310
muscles, and oxygen consumption in health and emphysema, 790
paralysis, in porphyria, intermittent, acute, 288
response of guinea pigs to sulfuric acid mist, 314
symptoms, tobacco smoking, and ventilatory capacity, 623
tract
lower, tumor-like amyloid of, 600
and lungs, in Asian influenza, fatal, 463
pH studies on, endotracheal aspirate of healthy newborns, 310
Reticuloendothelial system, reactivity of, in tuberculosis, human, treated and untreated, 482
Retractive syndromes, respiratory function in, 479
Rheumatoid disease and pulmonary lesions, 133
Ribs, "cough" fracture of, pneumothorax in, 767
Roentgenography
angiocardiography, in effusions, pericardial, massive, surgery in, 142
angiography
automatic injector for, 302
pulmonary, in tuberculosis, roentgenography in urinary tract after, 474
selective, in normal and pathologic lung segments, 474
in bronchiectasis, diagnosis of, 766
bronchography
and bronchoscopy in bronchial cancer, 610
preoperative, and postoperative bronchopleural fistula, 782
bronchotomography of bronchial tree, after lung resection, 142
campaign in Edinburgh, 298
densiography, pulmonary, fluoroscopic, 619
diagnostic, hazards of, physicists viewpoint, 783
electrokymography in pulmonary vessels in bronchial carcinoma, 128
in excavation of metastatic pulmonary nodules, 475
in fibrocystic disease of pancreas, 286
fluoroscopy in pediatric roentgenography, 302
in histoplasmosis, intrathoracic, 474
iconography of lung diseases, miliary, 142
in lupus erythematosus, systemic, and arthritis, rheumatoid, pleuropulmonary findings in, 619
mass
in Brazilian cities, 615
dual reading in, 299
and morbidity, 615
in sarcoidosis, 473
in tuberculosis, pulmonary, 615
in mediastinal borders, 302
in myocarditis, interstitial, primary, in Haifa, 474
negative results in, causing ignorance of bronchogenic carcinoma, 601
pediatric, fluoroscopy in, 302
in pneumonia in adults during 1957 influenza epidemic, 602
pre-, state of silicosis, studies of, 780
routine, tuberculosis diagnosed by, 467
in sarcoidosis, pulmonary, 143
stratigraphy and bronchoscopy in tuberculosis, bronchial, 302
survey
chest, of Amsterdam population, 141
mass, evaluation of, in tuberculosis and heart disease, 141
tomography, transverse, axial, of chest, 475
in tracheopathia osteoplastica, 303
transparencies in, for projection, Polaroid Land process in, 475
in tuberculous cavities, diagnosis of, limitations of, 783
in urinary tract after pulmonary angiography in tuberculous patients, 474
Rupture, traumatic, of bronchus, 292

Salmonella enteritidis infection, effect of BCG infection on resistance of mice to, 794
Sanatorium(s)
tuberculosis
long-term patients in, 617
pulmonary, patients in, antimicrobial results in, 304
tuberculous laryngitis in patients in, 135
Sarcoidosis. *See* Mycoses
Sarcoma. *See* Tumor(s)
Schistosome eggs in feces, variation of, after tartar emetic, 288
Schistosomiasis, acute, and cor pulmonale, acute, 605
Schönlein-Henoch purpura. *See* Purpura
Scleroderma
of esophagus, 466
of lungs, 604
Scotland, Edinburgh, roentgenography campaign in, 298
Sea View Hospital, principal causes of death at, 472
Septal defects, interatrial, intravascular carbon dioxide gas in, 309
Serum(a)
assay of free isoniazid in, vertical diffusion test in, 476
calcium, in sarcoidosis, intravenous calcium infusion test in, 127
inhibitory power of, after simultaneous administration of isoniazid and pyridoxine, 146
levels, maximal, of free isoniazid, in resistant strains, 305
proteins, paper electrophoresis of, in pulmonary tuberculosis, 468
Shotgun-blast injuries of chest, close proximity, 135
Shunt operation with arterial homotransplants in Fallot's tetralogy or tricuspid atresia, 611
Silica toxicity, neutralization of, *in vitro*, 780
Silicosis
and emphysema, respiratory dead space and alveolar ventilation in, 148
experimental, 150
pre-roentgenography stage of, studies of, 780
pulmonary
experimental, in rats with spontaneous tuberculous infection, 480
morphologic studies of, as immunologic disease, 480
simple, in retired miners, 781
Silicotuberculosis and tuberculosis, intraleukocytic ascorbic acid in, 133
Skin
reaction to histoplasmin in Campania, 297
tuberculosis, Vitamin C in, 469
Smoke and atmospheric deposit in cancer and bronchitis mortality, 613
Smoking (of tobacco), 299
lobeline as deterrent to, 771
respiratory symptoms, and ventilatory capacity, 623
Sodium salicylate in respiratory failure, 623
Soot in human lungs, disappearance of carcinogens from, 778
South America, Brazil, tuberculosis hospitals in, 473
Spinal meningitis, tuberculous, without cerebral meningitis, 608
Spleen transposited into thoracic cavity and the vena cava superior, collateral circulation between, 777
Sputum cytology, 482
Staphylococcus
empyema, 129
in infants and children, surgery in, 470
infections
drug-resistant, combined antimicrobials in, 620
hospital-acquired, 615
pulmonary, in adults, 288
in respiratory disease, 606
pneumonitis in surgery of thorax, 137
tension pneumatoceles, intracavitary suction tube drainage in, 293
Staphylococcus aureus, pneumonia from, 771
Status epilepticus, D-tubocurarine and controlled respiration in, 622
Stenosis
aortic, calcific, surgery in, in adults, 295
mitral
disseminated nodular pulmonary ossification in, 769
influenzal pneumonia in, 287
pulmonary, without ventricular septal defect, 623
tricuspid, clinical and hemodynamic findings in, 790
Sternum
bifid, autogenous cartilage in, 776
in syndrome of congenital defects, 126
Steroids. *See* Hormones
Stethoscope earpiece fit, anatomic variations of auditory canal in, 772
Stomach and lung, plasmacytoma of, 467
Strangulation, spontaneous, in diaphragmatic hernia, 470
Stratigraphy. *See* Roentgenography
Streptomycin
isoniazid
and kanamycin, in *M. tuberculosis* in mouse, 626
and PAS in tubercle bacilli in cells from two different sources, 793
in tuberculous cavities, 306
resistance
and isoniazid resistance, 306

of tubercle bacilli in tuberculosis, pulmomary, primary, nontreated, 300
resistant
and isoniazid-resistant tubercle bacilli, virulence of, 787
PAS-, and isoniazid-resistant tubercle bacilli, 792
in tubercle bacilli, 476
in tuberculosis, skeletal, 291
vestibular nerve damage from, pantothenic acid in, 787
Streptovaricin-isoniazid in tuberculosis, experimental, of guinea pigs, 144
Sulfone or PAS, and isoniazid, in tuberculosis, pulmonary, in East Africans, 785
Sulfuric acid. *See* Acid(s)
Superinone, 0.125%, in laryngotracheobronchitis, acute, 131
Surgery
in bronchiectasis, 294
in cavities, open-healing, 294
in drainage of pulmonary veins to right side of heart, 295
in effusions, pericardial, massive, angiocardiography in, 142
electro-encephalography during, 775
in enteritis, tuberculous, perforated, acute, 468
in funnel chest, 611
of heart, open, temporary cardiopulmonary bypass in, 294
intrathoracic, followed by acute gastric and duodenal ulcers, 138
oximetry during, 471
post-
congestive atelectasis as fatal complication, 774
hypoventilation, hypoxia, and acidosis, 469
in pulmonary conditions in childhood, 295
in pulmonary lesions, metastatic, 612
shunt with arterial homotransplants in Fallot's tetralogy or tricuspid atresia, 611
in staphylococcal empyema in infants and children, 470
in stenosis, aortic, calcific, in adults, 295
successful, in sarcoma, pulmonary, 613
of thorax, staphylococcal pneumonitis in, 137
in tuberculosis
pulmonary, 608
in adults, 472
electrocardiographic irregularities after, 612
relapse after, 612
in ventricular septal defects and severe pulmonary hypertension, 470
Sweden
influenza epidemic of 1957–1958 in, vaccination trials, 297
occupational mortality in 3 professions in, 614
Tartar emetic, effect o., on schistosome eggs in feces, 288
Tebafen® in tuberculosis, pulmonary, chronic, 308
Tenth National Congress of Tuberculosis, report to, on tuberculosis hospitals in Brazil, 473
Tessalon®. *See* Benzonatrate
Test(s)
conversion, in schoolchildren, after BCG vaccination, 138
corticosteroids in experimental tuberculosis in guinea pigs, 794
for drug-resistant tubercle bacilli, using culture media with multiple agents, 787
Galleria mellonella, in antimycotic drug study, 303
for histoplasmosis, using precipitin-in-gel reactions, 139
of hypertension, pulmonary, monolobar, acute, 788
intravenous calcium infusion, in sarcoidosis, 127
for kanamycin sensitivity of tubercle bacilli, 307
Kveim, in sarcoidosis and family contacts, 473
of liver perfusion, in isoniazid metabolism, 621
niacin, in differentiation of human tubercle bacilli, 311
peroxidase reaction, in population analysis of isoniazid-resistant tubercle bacilli, 312
production of pulmonary tuberculosis cavities in dogs, 793
pulmonary function screening-, in bronchial asthma, 308
of respiratory function, in chest hospital work, 789
for tracheal function, standardization of, 148
tuberculin
intradermic, Finnish, 616
nonspecific reactions from various PPD concentrations, 617
PPD, reactions to two concentrations of, 781
vertical diffusion
in assay of free isoniazid in serum, 476
in resistance of *M. tuberculosis* and serum isoniazid level, 306
1314 Th in tuberculosis, 788
Thiosemicarbazone, water-soluble, and venous perfusion of isoniazid, in tuberculosis, pulmonary, 306
Thoracoplasty
anesthesia in, 776
paravertebral, kinking of bronchus after, causing giant cyst in case of tracheal lobe, 136
in tuberculosis, pulmonary, 138
Thoracotomy
compression of superior vena cava during, electro-encephalography in, 777

emergency, for massive spontaneous hemopneumothorax, 777
nonobstructive consolidation-atelectasis following, 776
Thorax
cavity of, spleen transposited into; and vena cava superior, collateral circulation between, 777
surgery in, staphylococcal pneumonitis following, 137
Thorium inhalation from road dust on Niue Island, 473
Thromboembolism, postoperative, increased pulmonary megakaryocytes in, 612
Thrombosis
of pulmonary arteries, major, 136
venous, peripheral, and segmental pulmonary artery aneurysms, 458
Thymus, mediastinal cyst of, 128
Tobacco smoking. *See* Smoking
Tomography. *See* Roentgenography
Torulosis. *See* Mycoses
Trachea
ciliary streaming through, 481
subtotal excision of, and establishment of sternal tracheostomy, 471
function of standardization of tests for, 148
lobe of, cyst from, from kinking of bronchus after paravertebral thoracoplasty, 136
Tracheobronchial deformity in tuberculosis, pulmonary, patterns and dynamics of, 773
Tracheobronchial tree, human, bacterial flora of, 791
Tracheopathia osteoplastica, roentgenography in, 303
Tracheostomy, sternal, in subtotal excision of trachea, 471
Trauma, pulmonary, pre-existing pulmonary disease in, 604
Tricuspid atresia, shunt operation with arterial homotransplants in, 611
Trifluroethane
in acid-base balance, 610
vaporizers for administration of, 610
L-Triiodothyronine in tuberculosis, pulmonary, 477
Tryptophanase, isoniazid and its derivatives in, 785
Tubercle bacillus(i)
acetone-soluble lipids of, in antimicrobial activity of microphages, 626
air-borne droplet infection with, in experimental animals, 150
biochemistry of, for clinicians, 625
bovine, in man, during 1953–1957, 616
in cells from two different sources, streptomycin, isoniazid, and PAS in, 793
in the community, 300
drug-resistant
and postpneumonectomy complications, 787
tests for, 787
late excretion of, in tuberculosis, primary, 134
human
niacin test for differentiation of, 311
production of nicotinic acid by mycobacteria cultured on synthetic liquid-, solid-, and Dubos Tween®- albumin media, 312
hydrocortisone in intracellular growth of, 793
isoniazid-resistant
peroxidase reaction in population analysis of, 312
and streptomycin-resistant, virulence of, 787
kanamycin
streptomycin, acriflavine, 8-azaguanine, and Na azide in, 476
susceptibility of, 149
determination of, by direct and indirect methods, 307
malachite green-fuchsin staining of, 149
peroxidase reaction in, in isoniazid-resistance study, 792
polysaccharides of, immuno-precipitation of, in gel, 792
single-cell culture of, 792
streptomycin-, PAS-, and isoniazid-resistant, 792
from tuberculosis, pulmonary, primary, nontreated, streptomycin and isoniazid resistance of, 300
Tubercles, sarcoid, at site of tuberculin injections, 291
Tuberculin
adsorption of, to glass, 301
allergy, in geriatric patients, 133
desensitization, in keratoconjunctivitis, phlyctenular, recurrent, 773
human and avian sensitivity in Union of South Africa, 300
injections, sarcoid tubercles at site of, 291
and lepromin reactions, correlation between, and influence of BCG on nonreactors in leprosy contact children in Djakarta, 140
PPD
different concentrations of, nonspecific reactions from, 617
-S, various doses of, changing cutaneous reaction after BCG vaccination, 778
test, reactions to two concentrations of, 781
purified
stability of, in high dilution, 300
standardization of, for international use, 301
reactions
in dogs, 150
re-evaluation of examination of, 300
RT 23, purified, preparation of, 301
sensitivity, in healthy and tuberculous persons, 616
stabilization of, diluents for, 301

survey, in British West Indies, 616
test
intradermic, Finnish, 616
nonspecific reactions from various PPD concentrations, 617
in tuberculosis, experimental, in guinea pigs, 481
therapy, of mouse thigh tuberculosis, 150
Tuberculoma. *See* Tumor(s)
Tuberculosis
adrenocorticosteroids in, 602
in animals, domestic, public health aspect of, 141
anti-, antibodies in school children, 778
avian, in the human, 468
and branchial cysts, lateral, 290
bronchial
bronchoscopy and stratigraphy in, 302
chemotherapy in, 772
steroids in, 133
chemotherapy in, present status of, 619
childhood
bone marrow and peripheral aspirates in bacteriologic diagnosis of, 313
in Hungary, 301
lobectomy in, 137
resection, pulmonary, in, 138
cycloserine in, 304
diagnosed by routine roentgenography and clinical symptoms, in French Armed Forces in Germany, 467
diseases of, from different species of acid-fast bacilli, 624
ethionamide in, 788
experimental
in golden hamster, 151
in guinea pigs
corticosteroids in, 794
isoniazid-streptovaricin in, 144
tuberculin test in, 481
of mice, kanamycin in, 626
of mice and guinea pigs, kanamycin and other drugs in, 307
gastric, and duodenal ulcer, 291
of genital tract, menstrual blood in diagnosis of, 134
genitourinary, infectiousness of, in women, 291
and health, tuberculin sensitivity in, clinical significance of, 616
and heart disease, mass surveys in, evaluation of, 141
hematogenous, with leukemoid reaction, 774
hospitals, in Brazil, 473
human
kanamycin in, 476
treated and untreated, reactivity of reticuloendothelial system in, 482
infection with
of infants, by cured patients living in home, 473
reactivation in lymph nodes of guinea pigs treated with isoniazid, 305
inflammation from, corticosteroids in, 482
in Japan, current status of, 618
kanamycin in, *in vitro* studies on, 307
laryngeal, 609
causing laryngitis, in sanatorium patients, 135
miliary, adrenocorticals in, 144
of mouse thigh, tuberculin therapy in, 150
nonhospitalized patients with, drug trend in, 619
in a nurses' school, 617
ovarian, disseminated, acute, unilateral, 609
of pancreas, antimicrobials in, 774
primary
in children, 144
ingestion, 608
late excretion of tubercle bacilli in, 134
prophylaxis, 144
chemotherapy or BCG vaccination in, 617
isoniazid and BCG in, 617
pulmonary
acid-fast bacilli, atypical, in, 149
in adults, surgery in, 472
alpha-ethyl-thioisonicotinamide in, 620
angiography in, roentgenography in urinary tract after, 474
antimicrobials in, immediate and long-range results of, in sanatoriums, 304
antistreptolysin titer in, 772
aorta, thoracic, rupture of, in, 290
in the Bantu, urban, 468
and carcinoma
bronchogenic, simultaneous occurrence of, 772
of lung, 607
chronic
blood lactic and pyruvic acids and cardiorespiratory function in, 309
drug-resistant, in Kenya, prednisolone in, 620
pathologic cardiac findings in death from, 482
respiratory function in, 790
Tebafen® in, 308
cor pulmonale in, 773
drug-resistant
cycloserine and pyrazinamide in, 145
treatment of, 787
endoscopy in bronchial changes in, 138
glucuronate of isoniazid in, 785
heterophile antibodies in, 467
intrapleural pneumothorax and pneumoperitoneum in, 776
isoniazid-pyrazinamide in, 622
kanamycin in, 786

lymphatic submucous endobronchial route in contralateral diffusion of, 313
nonacid-fast bacilli in, 781
PAS in, goiter and myxedema from, 145
pneumonectomy in, pulmonary function after, 790
primary, nontreated, streptomycin and isoniazid resistance in, 300
proteinemia in, 290
pyrazinamide in, 477
and cycloserine in, 145
and isoniazid in, 786
rehabilitation, professional and social, in, 301
relapse of
chemotherapy, long-term, in, 145
after completion of chemotherapy in, 784
after surgery in, 612
resection in, 472, 612
roentgenography, mass, in, 615
in Salisbury, 782
serum proteins in, paper electrophoresis of, 468
surgery in, 608
electrocardiographic irregularities after, 612
thoracoplasty in, 138
tracheobronchial deformity in, 773
L-triiodothyronine in, 477
uropepsinogen excretion in, 290
venous perfusion of isoniazid and water-soluble thiosemicarbazone in, 306
viomycin pantothenate-sulfate in, 308
sanatorium, long-term patients in, 617
and silicotuberculosis, intraleukocytic ascorbic acid in, 133
skeletal
isoniazid and rehabilitation in, 621
streptomycin in, 291
of skin, Vitamin C in, 469
treatment of, in general hospital, 142
of uterus, 135
D-Tubocurarine, in status epilepticus, 622
Tularemia, diagnosis of, 782
Tumor(s)
chemodectoma, mediastinal, 289
endothelioma, primary, of pleura, 606
granuloma
childhood disease from, fatal, new syndrome, 771
chronic disease from, in pulmonary infiltration, indeterminate, 475
eosinophilic, of lung, 130
hamartochondroma, endobronchial, 289
hemangioma, sclerosing, circumscribed, of lung, appearing as "coin" lesions, 466
hygroma, cystic, 777
hypernephroma, as continuous chest murmur, 131
intrathoracic, dumbbell-shaped, and paraplegia of lower extremities, 606
leiomyoma of esophagus, 772
-like amyloid of lower respiratory tract, 600
of lung, malignant, presenting as cystic formation, 128
mesothelioma, multiple, resectable form of, 612
neoplasm
benign, of lung, 466
bronchial, primary, radiation therapy in, 143
plasmacytoma of lung and stomach, 467
sarcoma, pulmonary, successful surgery in, 613
trophoblastic, and choriocarcinoma in women, chemotherapy of, 128
tuberculoma-like shadows, chemotherapy in, 773

Ulcer(s)
duodenal, and gastric tuberculosis, 291
of esophagus, 606
gastric
acute, and duodenal, after intrathoracic surgery, 138
chest pain in diagnosis of, 606
peptic, and emphysema, pulmonary, 129
United States
cancer of lung, death rates from, 139
echinococcal disease in, 296
Urinary tract, roentgenography in, after pulmonary angiography in tuberculous patients, 474
Uropepsinogen, excretion of, in tuberculosis, pulmonary, 290
Uterus, tuberculosis of, 135

Vaccination
BCG. *See* BCG vaccination
in influenza
epidemic of 1957–1958 in Sweden, 297
pandemic of 1957–1958, 603
Vaccine, tuberculosis, irradiated, in leprology, 479
Vagotomy and pneumothorax in dogs, pulmonary circulation after, 478
Vanadium poisoning, 473
"Vanishing lung" and unilateral emphysema, 604
Vaporizers in trifluroethane administration, 610
Vein(s)
pulmonary
drainage of
partial, anomalous, 137
to right side of heart, surgery in, 295
Vena cava
superior
compression of, during thoracotomy, electroencephalography in, 777
obstruction of, 289
and spleen transposited into thoracic cavity, collateral circulation between, 777
syndrome of, 607

Ventilation
anomalies of, during anesthesia, 788
manual versus automatic, in elastic recoil of lung, 310
pulmonary, regulation of, in pulmonary emphysema, 478
and respiratory muscle activity, airway resistance in, 310
Ventricular septal defect and severe pulmonary hypertension, temporary artificial ductus in surgery of, 470
Vertical diffusion test. *See* Test(s)
Vessel(s)
changes of, and pulmonary hypertension in congenital heart disease, 623
peripheral, resistance of, alterations in, in immersion hypothermia, 794
pulmonary, in bronchial carcinoma, electrokymography in, 127
Vim-Silverman needle in pleural biopsy, 610
Viomycin
hypersensitivity, 146
–pantothenate-sulfate in tuberculosis, pulmonary, 308
Virulence, quantitative studies of, 151
Virus(es)
adeno-, causing pneumonia, 465
Coxsackie, infections with, varied clinical signs of, 467
hemadsorption
in childhood, 618
in respiratory illness in childhood, 618
influenza A (Asian strain) isolation from influenza patients with pulmonary complications, in 1957–1958 pandemic, 603
type A/Asia/57, influenza pandemic in Norway from, 297
70, newly recognized, in man, 618
Vital capacity
fast, and various timed expiratory capacities, relationships between, 788
values, normal, and maximal expiratory volume per second, 311
Vitamin B_6 enzymes, isoniazid and its derivatives in, 785
Vitamin C in tuberculosis of skin, 469

Wales, lung cancer mortality in, 139
Wegener's granulomatosis, 133

X-ray. *See* Roentgenography
X-ray therapy. *See* Radiation therapy